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Effects Of Statin Drugs And Tocotrienol Rich Fraction Supplementation In Chronic Hemodialysis Patients And Metabolomic Profile

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EFFECTS OF STATIN DRUGS AND TOCOTRIENOL RICH FRACTION SUPPLEMENTATION IN CHRONIC HEMODIALYSIS PATIENTS AND METABOLOMIC PROFILE

by

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_____

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DEDICATION

This thesis is dedicated to the significant people in my life:

Benereta and Sulejman Latifi

My beloved parents who never failed to support me through all my trials and tribulation in life. I would like to thank them for their sacrifices, immigrating to the United State so my brother and I could have better lives for ourselves. At the same time I would like to thank them for the life lessons they taught me, financial support and never giving up in believing in my dreams.

Ivi Latifi

My beloved brother, who is always there for support, believing in my dreams and goals in life.

Ferit and Sadet Shehu; Shyqeri and Jalldyz Latifi

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	:	Amino Acids
ApoA1	:	Apo-Lipoprotein A1
B.P.		Blood Pressure
BMI	:	Body Mass Index
CETP	:	Cholesteryl Ester Transfer Protein
CKD	:	Chronic Kidney Diseases
CRP	:	C - Reactive Protein
CVD	:	Cardiovascular Disease
eGFR	:	Estimated GFR
ESRD	:	End Stage Renal Disease
GFR	:	Glomerular Filtration Rate
Hb	:	Hemoglobin
HD	:	Hemodialysis
HDL-C	:	High Density Lipoprotein Cholesterol
IDL	:	Intermediate Density Lipoprotein
IL-6	:	Interleukin-6

IQR	:	Interquartile Ranges
KDIGO	:	Kidney Disease Improving Global Outcomes
KDOQI	:	Kidney Disease Outcomes Quality Initiative
Kt/V	:	Dialyzer Clearance x Time Over Volume Distribution of Urea
LDL-C	:	Low Density Lipoprotein Cholesterol
MDA	:	Malondialdehyde
MVDA	:	Multivariate Data Analysis
NFκB	:	Nuclear Factor Kappa B
NMR	:	Nuclear Magnetic Resonance
PCA	:	Principal Component Analysis
PEW	:	Protein Energy Wasting
PLS	:	Partial Least Square
PLS-DA	:	Partial Least Square – Discriminant Analysis
RCT	:	Randomized Controlled Trial
RRT	:	Renal Replacement Therapy
SCr	:	Serum Creatinine
SD	:	Standard Deviation

SEM	:	Standard Error of Mean
Ser. Alb	:	Serum Albumin
SR-B1	:	Scavenger Receptor Class B-1
SREBPs	:	Sterol Regulatory Element Binding Protein
TAG	:	Triacylglycerol
TAP	:	Total Antioxidant Power
TC	:	Total Cholesterol
TNF- α	:	Tumor Necrosis Factor Alpha
TP	:	Tocopherol (Vitamin E)
TRF	:	Tocotrienol-Rich Fractions (Vitamin E)
TT	:	Tocotrienol
USRDS	:	US Renal Data System
VLDL	:	Very Low Density Lipoprotein
WHO	:	World Health Organization

CHAPTER 1

INTRODUCTION

Chronic Kidney Disease and End-Stage Renal Disease

Chronic kidney disease (**CKD**) is a heterogeneous disorder which currently is on the rise and has been classified as a serious public health issues in the United States and worldwide. The disease effects both kidneys, and is defined as a progressive decline in kidney structure and functions. If present for more than three months, it has serious implications for patient health [1]. In the United States the disease effects more than 10% of adults (more than 20 million people). Based on a National Health and Nutrition Examination Survey (NHANES) between 1988-2008 the prevalence of CKD has grown most rapidly in people of ages 60-70 years and older jumping from 18.8 to 26.0 percent (NKUDIC) [2, 3]. In simplistic terms, this condition is one in which the kidneys are damaged to a point where the organs cannot filter blood to maintain adequate living for the patient. Individuals who are considered to be at risk for CKD, are generally adults with diabetes or high blood pressure, or both. Approximately 1 of 3 adults who have diabetes and 1 of 5 adults with high blood pressure have some form of CKD in the United States according to CDC investigation [4]. Other noticeable risk factors for CKD include cardiovascular disease, obesity, high cholesterol, lupus, and family history of CKD. The risk of developing CKD further increases with age; while CKD is more common among women, it is estimated that men with CKD are 50% more likely than women to progress into kidney failure [4].

Testing for Kidney Disease

The damage due to various kidney diseases can be asserted medically by looking at patient's glomerular filtration rates (**GFR**). A GFR below 60 mL per min for more than 3 months or when patient's urine albumin-to-creatinine ratio is over 30mg/g, classifies these individuals to be suffering from CKD [1, 2] refer to **Table 1-1**. Newer methods to calculate renal functions, also use estimated GRF (eGFR), in which case only a measurement of creatinine levels in the blood is needed. An eGFR of 90 or above is considered to be normal. Dialysis or kidney transplant is required at an eGFR of < 15mL/min **Table 1-1**.

Treatment Methods

CKD patients with ESRD have three treatment options; 1) hemodialysis, 2) peritoneal dialysis or 3) a kidney transplant. Hemodialysis (HD) utilizes a man-made membrane (dialyzer or hemodialyzer) in order to remove waste products from the blood (e.g. urea), it restores proper balance of electrolytes in the blood and eliminates extra fluids from the body. This treatment usually occurs in the presence of health care professionals in the hospital or clinics usually done 3 days (or every other day) a week and 3-5 hours a day [5]. Certified dialysis practitioners create a fistula connected to one of the arteries, which connects to the veins in the lower arm in order to obtain access to the blood circulatory system so dialysis can proceed [5]. Peritoneal dialysis uses a membrane inside the patient's body (peritoneal membrane) as the filter to clean wastes and extra fluid from the body and returns the electrolyte level to normal. This is a process that does not requires in-center treatment like hemodialysis does, and can be performed at a home setting so long

as a catheter is placed into the bell [6]. The last treatment for CKD patients is to obtain a new kidney via a transplant. It is a procedure which requires surgery and a donated kidney, other from a living related relative, unrelated donor (friends) or a deceased donor. The various options are shown in **Figure 1-1**.

CKD and ESRD

End-stage renal disease (**ESRD**) occurs when the (eGRF <15 mL/min/ 1.73 m^2) (Stage 5 of CKD), when both organs are in a total or permanent failure. When the kidneys do fail, the body retains fluids and harmful wastes build up. Once diagnosed with ESRDS, CKD patients need renal replacement therapy (RRT) to replace the work performed by the failing kidneys in order to sustain life [2]. Currently the most common RRT in the US is hemodialysis (HD) which accounts for 98% of the total cases of patients with ESRD [1]. In the USA incidents of ESRD increased steadily from 1980-2001 but they seem to be leveling off by approximately 350 per million population (pmp) [7]. It was estimated that in 2011, roughly 113,136 patients in the USA began treatments for ESRD, and of those patients 7 of 10 new cases, had prior involvement or complication with diabetes and hypertension. Diabetes mellitus (DM) and hypertension are two of the leading causes of ESRD. Other disease which lead to ESRD includes, autoimmune disease (lupus erythematous associated nephritis), drug toxicity, glomerular disease (caused by hepatitis B, C, and HIV viruses) and tubular interstitial nephritis [7].

Comorbid Conditions

Chronic kidney Disease (CKD) patients with ESRD are known to have systemic oxidative stress which puts them at high risks for the development of cardiovascular disease

(CVD), which is currently the leading cause of morbidity and mortality in this population [8]. ESRD patients, on chronic HD also experience an accelerated form of atherosclerosis, which is induced by inflammation, impairment of antioxidant system and elevated oxidative stress. Patients' who incur ESRD; show evidence of increasing risks of hospitalization, complications from cardiovascular disease, malnutrition, and chronic inflammation [9, 10]. The alarmingly increase, in rates of cardiovascular complications like cardiovascular disease (CVD) currently accounts for 50% mortality rates in ESRD patients which are estimated to have a 15 to 30 times higher chance than the general age-matched population to experience a cardiac event [9, 11]. One of the most common forms of CVD that ESRD patients experience include acute myocardial infractions, and different forms of atherosclerotic vascular dieses (e.g. stroke, chronic coronary artery disease and transient ischemic attacks) [11]. Atherosclerosis is an inflammatory process initiated by many factors including accumulation of low-density lipoprotein (LDL) in the arterial walls **Figure 1-2**. Elevated plasma LDL after exceeding a certain threshold (high LDL levels), enters the arteries at a fast pace, at which point removal occurs at slower rate, and this leads to accumulation. This LDL is one of the contributors to atherosclerosis [12, 13].

Comorbidities to ESRD, can involves organs other than the kidneys which may also be responsible for renal failure (e.g. diabetes mellitus, myeloma) [14]. In ESRD patients comorbidities include inflammation, oxidative stress and protein-energy wasting (PEW) [15]. Oxidative stress is referred to as a situation in which pro-oxidants overwhelm antioxidant defenses, resulting in increased biomarkers of oxidative damage and the accumulation of free radicals such as reactive oxygen species (ROS) and nitrogen species (RNS) [16]. In retrospect oxidative stress is linked to CVD because it is involved in the

modification of LDL, which instigates a cascade of reactions including adhesion of circulating monocytes on endothelial cells, monocytes migrating into arterial intimal, platelets activation and expression of various tissue factors by endothelial cells [17]. A highly prevalent comorbid condition that effects ESRD population is PEW which is known to be a very strong predictor for adverse outcome and mortality. PEW is characterized by the loss of muscle mass, weight loss (unintentional), a significant decline in nutritional parameters such as albumin, pre-albumin [18, 19] derangements of adipose tissues, and gastrointestinal, hematopoietic and immune systems issues due to deficiencies of multiple micronutrients. Other effectors which add to the increase in mortality rates of CKD patients with ESRD due to PEW are hypoalbuminemia, low serum cholesterol levels, low body mass index a reduced dietary protein intake [19]. The low serum albumin that is present in the ESRD population due to inadequate energy and protein intake, leads to loss of amino acids during dialysis and to non-compliance to fluid restriction. Albumin contains thiol moieties, an important antioxidant in plasma, so low plasma albumin correlates with low plasma total antioxidant capacity which than links with cardiovascular mortality in ESRD patients with hypoalbuminemia [20]. PEW furthermore is linked to persistent malnutrition and inflammation, which is responsible for a cascade of reaction that ultimately causes an increase in resting energy expenditure, loss of muscle mass and oxidation [21] **Table 1-2**.

Thus dietary intervention maybe potentially beneficial for CKD patients with ESRD in order to improve comorbid conditions, like inflammation, oxidative stress, PEW which seems to be effectors that accelerate CVD's and increase mortality. So far a limited number of dietary intervention studies in ESRD patients have focused on foods/supplements with anti-inflammatory and antioxidant effects as a means to decreasing

the various comorbidities. The study "Secondary Prevention with Antioxidants of Cardiovascular Disease in End State Renal Disease (SPACE)," demonstrated that supplementation with 800 IU of vitamin E per day decreased the overall mortality and morbidity based on composite cardiovascular end points [22]. Other studies involving omega-3 fatty acids in HD population have demonstrated that these nutrients possess beneficial anti-inflammatory, antioxidant and lipid altering properties in-vivo, in-vitro and clinical settings [23-25].

Potential Nutritional Agents to Reduce Comorbid Conditions:

Vitamin E Tocotrienol

Vitamin E encompasses different isomers. They are composed of two categories - tocopherols and tocotrienols. The four saturated analogues alpha (α-), beta (β-), gamma (γ-) and delta (δ-) are known as tocopherols (**TP**) which consist of a chromanol ring 15-carbon (phytyl) tail. The other forms of vitamin E are the four unsaturated (α-, β-, γ-, and δ-) analogues referred to as tocotrienols (**TT**) which possess a chromanol ring with three trans-double bonds toward the end of their farnesyl isoprenoid tail, distinguishing TT from TP [26, 27] **Figure 1-3**. Biological half-life of TT is considerably shorter than TP (by 4.5 to 8.7 fold). Vitamin E tocotrienols are compounds which are lipid rich plant products, natural and found in select vegetable oils. A good source for TT can be found in cereal grains exemplified by oat, barley and rye, while natural sources of TT are found in annatto, palm oil, and rice bran oil [27] **Figure 1-4**.

The structural differences of TT, three unsaturated bonds on the side chain may accounts for why tocopherols do not have the cholesterol-lowering properties which are in

TT. This is due to the fact that tocotrienols down-regulate 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase activity which happens to be the rate limiting activity in the mevalonate pathway that contributes to the synthesis of cholesterol [27]. TT have also exhibit cardio protective effects including raising serum HDL-C, and inhibiting oxidation of LDL, plaque instability, thrombogenesis, platelet aggregation, monocyte adhesion and various other cardiovascular dysfunction [27]. The absorption mechanism for all 8 isomers vitamin E is described to be similar as the lipid molecules, which means vitamin E is incorporated into mixed micelles and up taken by enterocytes. This uptake of vitamin E and mixed micelles are hypothesized to occur via simple passive diffusion as well as through intestinal scavenger receptor class B type I (SR-B1) [28]. Unfortunately the cellular mechanism for intracellular trafficking of vitamin E to be elucidated [28].

Chemically vitamin E functions as an antioxidant. Both tocopherols and tocotrienols have antioxidant activity due to their ability to donate a hydrogen atom (proton plus electron) from the hydroxyl groups on the chromanol ring, to a free radical in the body. However lately TT have also gained increasing attention due to indication of them possessing additional therapeutic properties [26]. Some potential therapeutically effects of TT are summarized in **Table 1-3**. A recent study on type 2 diabetic rats with (TRF) showed the activity of antioxidant enzymes (i.e. superoxide dismutase, and glutathione peroxidase) was restored and malondialdehyde (MDA), an oxidative stress marker, were reduced when compared to the untreated groups [29]. Effects of tocotrienol are also seen in the anti-inflammatory arena, where there are several lines of evidence that TT could block activation of NFκB, a family of transcription factors which play central role in regulation of genes critical for inflammation and immunity[30]. TRF is also described to obtain the

ability to effect in reducing IL-6, cytokines activity and inhibits LPS-induced secretion of TNF, further up/down regulation TT targets can be observed by **Figure 1-5** [26].

Statin Drugs Effects

This clinical intervention with TRF supplementation unlike other studies uses humans for subjects, of which are faced by the daily scrutiny of living with renal disease. Because of the multi-factorial affects that HD patients are face by, few subjects in this study were allowed to continue with their use of the statins medication (previously prescribed by their physician) in addition to TRF supplementation **Figure 3-1**. In general when statin drugs are used by these patients, metabolic pathways are affected. In specifics the drugs effect HMG-CoA reductase, were inhibitors (e.g. statin) use has been associated with reduced mortality from CVD's in patients with ESRD [31]. Studies have shown that when combining gamma-tocotrienols and statin drugs together on human malignant mesothelioma cells, a synergistic effect has been observed to associate and affect the outcome of the treatment for better. This combination effects was confirmed to be mediated by the inhibition of the mevalonate pathway, and effect were shown from apoptosis against malignant mesothelioma cell [32].

Rationale of the Study

Even though much knowledge has been obtained throughout the years in understanding the complex pathophysiology on what causes CVD complications in CKD patients with ESRD, unfortunately multifaceted clinical intervention aimed at improving CVD outcome in HD patients have been unsuccessful in solving the complete puzzle. This is evident from the fact that around two thirds of dialysis patients die within five year of having dialysis procedure encore, even though technology, medical techniques, and drugs

have improved drastically [33]. When it comes to mortality rates in ESRD patients in the United States, it is estimated to be as high as > 20% per year which is worse than cancer deaths [34].

Since the problems effecting ESRD patients are multifactorial issues, the objective of this study is to investigate the effects of supplementing with vitamin E-tocotrienol rich fraction (TRF), a micronutrient which has anti-inflammatory, antioxidant, and lipid lower capabilities into tackling these comorbid conditions experienced by this population. Therefore the aims of this investigation will be to explore changes in lipid profiles, inflammatory markers, and oxidative status as well as look into any changes in ESRD metabolomic profiles. Because of previous studies on human malignant mesothelioma cells, will explore if there are any synergistic effects found by supplementing TRF and statin drugs together when analyzing lipids and metabolite profiles in ESRD patients.

Hypothesis

It is hypothesized that by supplementing with tocotrienol rich fraction (TRF) vitamin E, for 16 weeks in ESRD patients undergoing hemodialysis it may help reverse and/or improve, oxidative status, inflammatory markers, increase antioxidants status and improve lipid profiles.

Table 1-1. GFR Categorical Classification

GFR Category	GFR (ml/min/1.73 m2)	Terms
G1	≥90	Normal or high
G2	60-89	Mildly decreased*
G3a	45-59	Mildly to moderate
G3b	30-44	Moderately to severe
G4	15-29	Severely decreased
G5	<15	Kidney failure

CKD- Chronic Kidney Disease; GFR- Glomerular Filtration Rate.

Current CKD staging based on GFR kidney function as is described by the guidelines of KDIGO 2013. In the absence of evidence kidney damage, neither GFR category G1 nor G2 fulfill the criteria for CKD [1].

Table 1-2. PEW effects on ESRD

Causes	Notes
1. Decreased protein and energy intake	a.Anorexia - Dysregulation in circulating appetite mediators - Hypothalamic amino acid sensing - Nitrogen-based uremic toxins b.Dietary restrictions c.Alterations in organs involved in nutrient intake d.Depression e.Inability to obtain or prepare food
2. Hypermetabolism	a.Increased energy expenditure - Inflammation - Increased circulating proinflammatory cytokines - Insulin resistance secondary to obesity - Altered adiponectin and resistin metabolism b.Hormonal disorders - Insulin resistance - Increased glucocorticoid activity
3. Metabolic acidosis	
4. Decreased physical activity	
5. Deceased anabolism	a.Decreased nutrient intake b.Resistance to GH/IGF-1 c.Testosterone deficiency d.Low thyroid hormone levels
6. Comorbidities and lifestyle	Comorbidities (diabetes mellitus, CHF, depression, coronary artery disease, peripheral vascular disease)
7. Dialysis	a.Nutrient losses into dialysate b.Dialysis-related inflammation c.Dialysis-related hypermetabolism d.Loss of residual renal function

CKD- Chronic Kidney Disease; ESRD- End-Stage Renal Disease.

A list of potential causes of protein energy wasting (PEW) on CKD patients' with ESRD population [21].

Table 1-3. Vitamin E tocotrienols properties

Tocotrienols properties	Remarks
1. Antioxidant	• TTs are more potent radical scavenger and has greater antioxidant activity again lipid peroxidation in liposome than TPs. • TT is more effective in the protection of cytochrome P450 against oxidative damage when compared to TP. • This is due to faster cellular uptake; faster recycling from the respective chromanoxyl radical forms in liposomal membrane and lipoproteins; higher inter-membrane mobility.
2. Anti-inflammatory	• ↓ inflammatory cytokines (CRP, TNF-α, IL-4, IL-6, IL-8, NFκB) • ↓ generation of reactive oxygen species • ↓ arachidonic acid derived eicosanoids
3. Lipid altering effects	• Hypocholestrolemic effects is unique to TT not TP • This is due to post-transcriptional suppression of HMG-CoA reductase protein; enhance ubiquitination of HMG-CoA reductase.
4. Effects on suppression, regression and progression of atherosclerosis	• Suppression of atherosclerosis is related to reduction in oxidative stress, total cholesterol, increased in HDL-C; ↓ adhesion molecules expression (e.g. VCAM-1, ICAM-1) • Do not regress atherosclerosis in experimental animals • Conflicting results in progression of atherosclerosis.
5. Antithrombogenesis	• δ-TT is a potent inhibitor of platelet aggregation. • TT reduced serum levels of thromboxane-B2 and platelet factor 4

(Sources: Frank et al., 2012; Prasad 2011; Vasanthi et al 2012)

TT- tocotrienol; TP- tocopherols; CRP- C-reactive protein; IL- interleukin; TNF- α- tumor necrosis factor alpha; VCAM-1- vascular cell adhesion molecule 1; ICAM-1- intercellular adhesion molecule 1; NFκB- nuclear factor kappa B; HDLC- high density lipoprotein cholesterol.

This show the potential mechanism of tocotrienols in reducing risk for CVD's. This depicts the contributing points, of antioxidant, anti-inflammatory, lipid altering, suppression of atherosclerosis and anti-thrombogenesis.

Figure 1-1. Treatment modles for CKD pateints with ESRD.

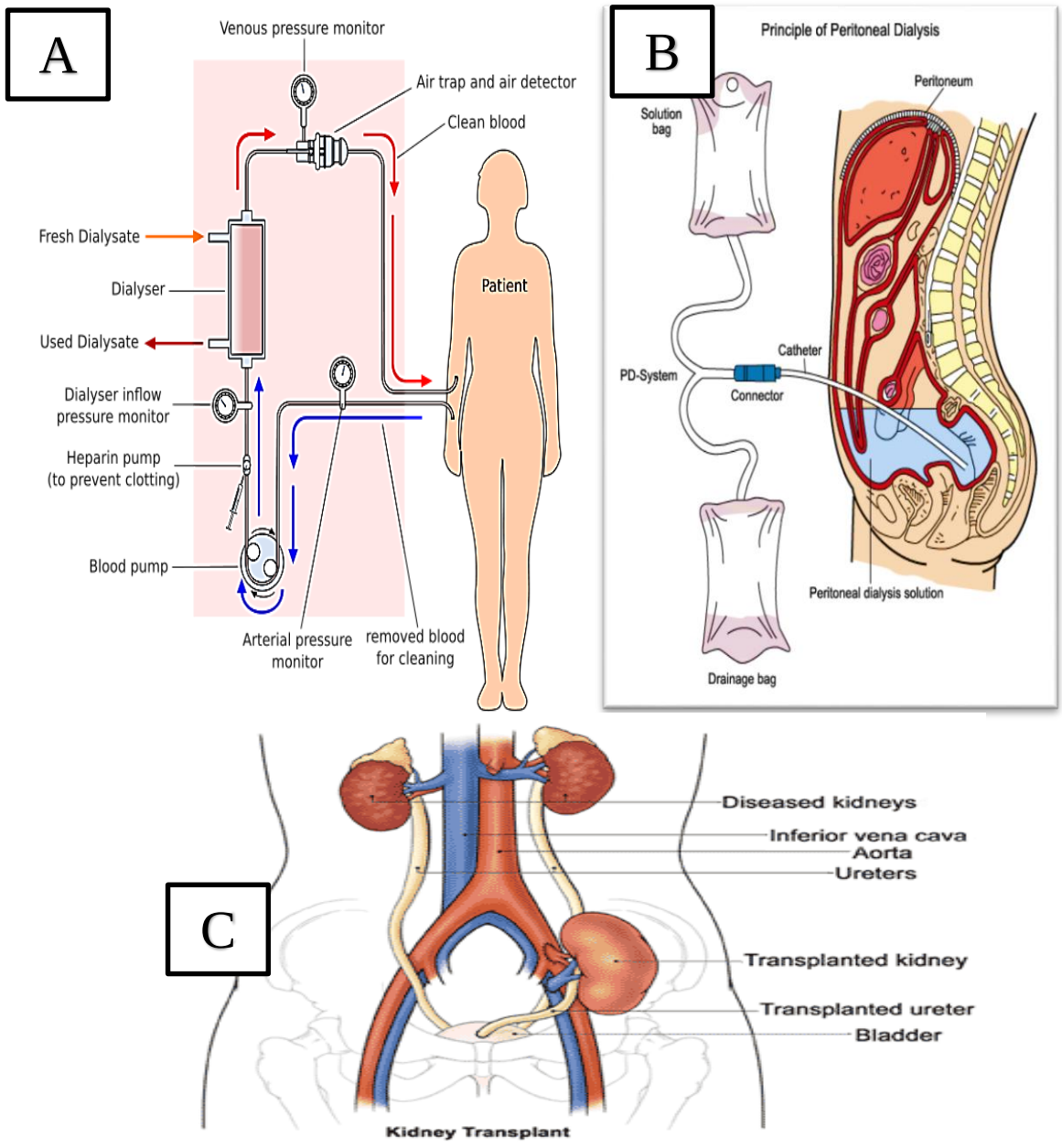
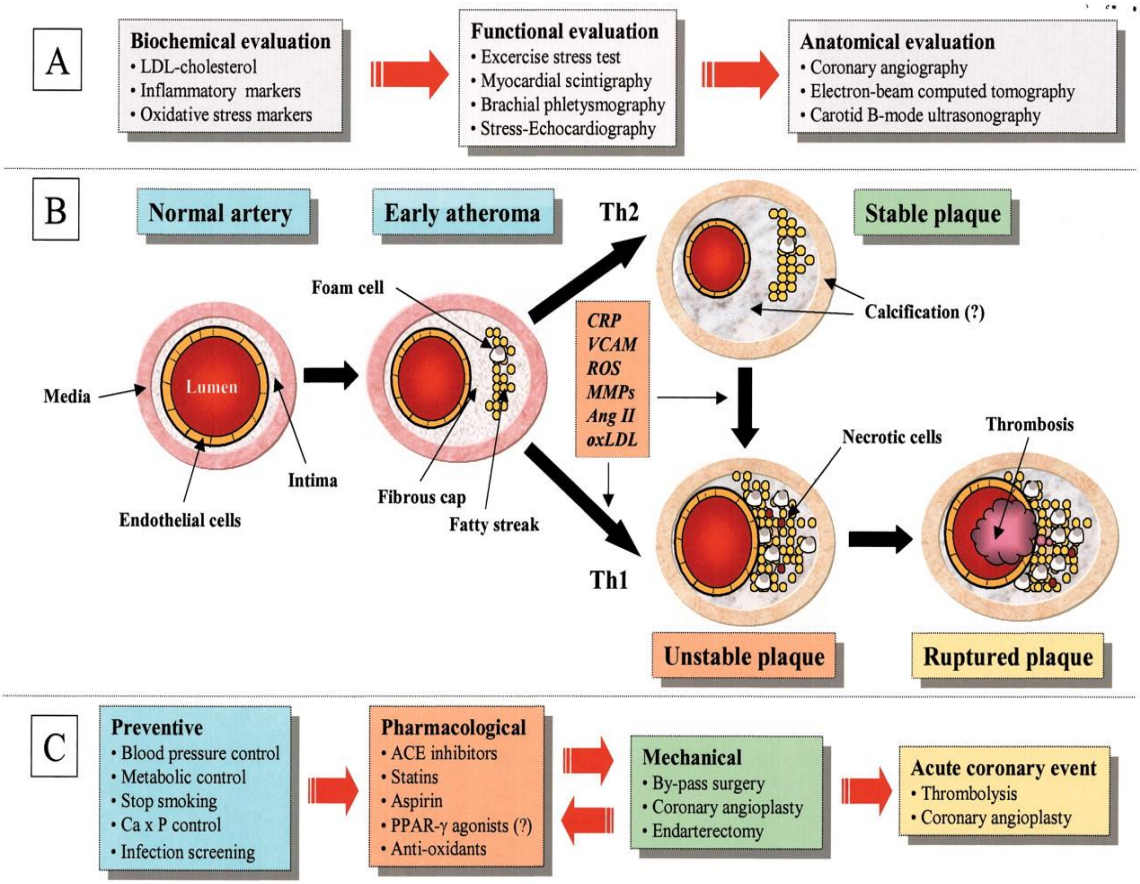


Figure **A**) Hemodialysis; **B**) Peritoneal Dialysis; **C**) Kidney Transplant.

A) Hemodialysis model the patients blood is pumped out of the body and passed through a dialyser membrane which allowes for diffusion of fluids and other waste substances across the semipermeable membreane (most common treatment in USA). **B**) Peritoneal dialysis uses the patients own peritoneum membrane to filter out fluids and other dissolved substances. **C**) A kidney from a donor is transplated in the iliac fossa, in the recipients contra lateral side. (Source: NKUDIC, 2013)

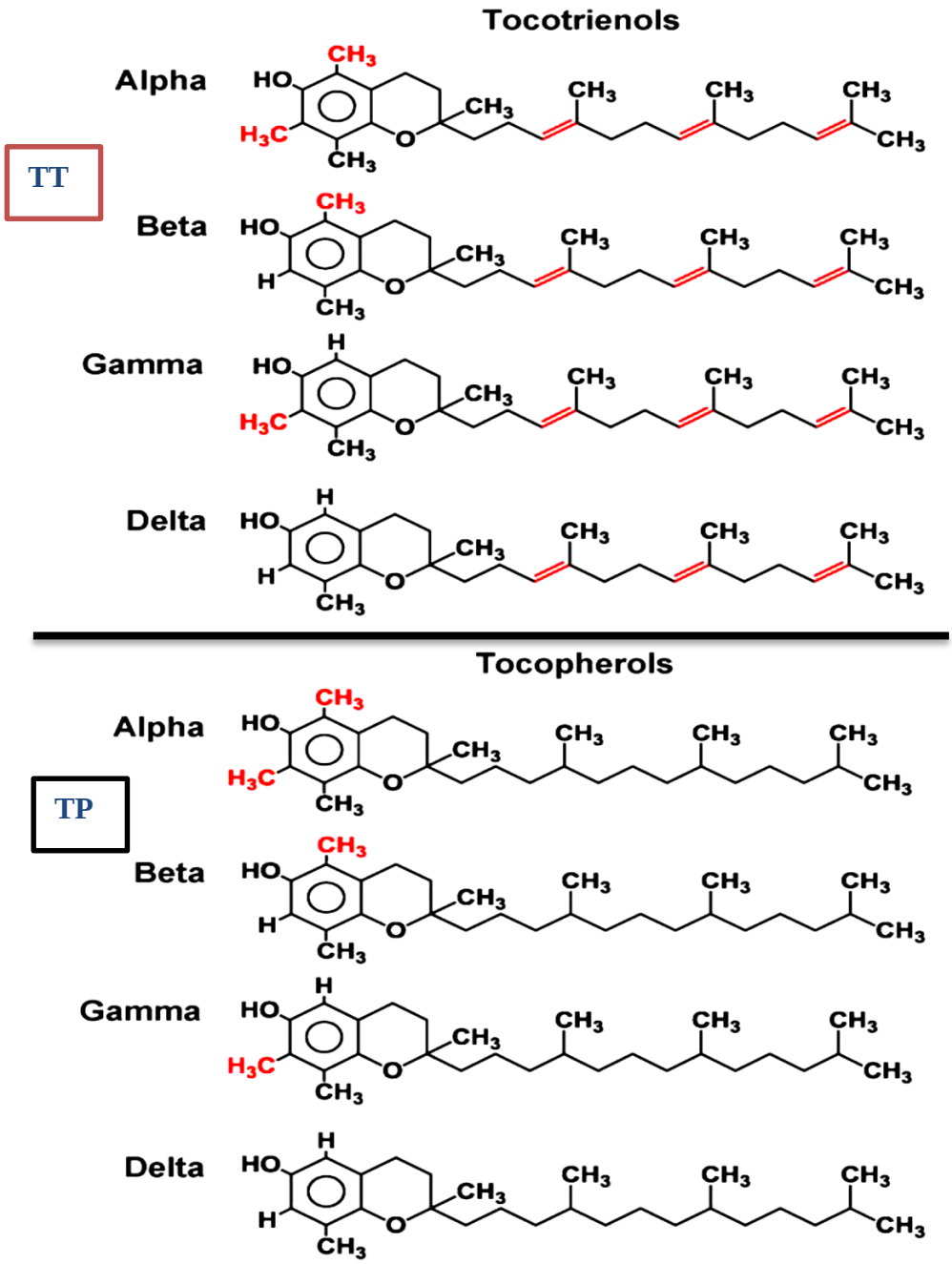
Figure 1-2. Biochemical functions ESRD patients experience leading to CVD's



Th- T-helper cell; CRP- C-reactive protein; VCAM- vascular cell adhesion molecules; ROS- reactive oxygen species; MMPs- matrix metalloproteinase; AngII- angiotensin II; ox-LDL- oxidized low-density lipoprotein

A) Depicts the biochemical, functional and anatomic evaluation of coronary heart disease in ESRD patients. **B**) Show the atheroma's development processes. Depicts the normal 3 layers of cell arteries who progress into early atheroma. Early artherogenesis, fatty streak is developed as a result of recruitment of inflammatory cells and foam cells (from accumulated lipids). When dyslipidemia and inflammatory conditions persist, lipid pool increases. Activated leukocytes secrete proteinase which caused degradation of the extracellular matrix and ultimately cell death. Th1 is stimulated by the presence of inflammatory cytokines that limits the synthesis of new collegen and may thin fibrous cap which may lead to rapture. Th2 activity leads to a more stable plaque and less susceptibility to rupture. **C**) Depicts certain therapeutic measures to be implemented according to different evolution phases of the atherosclerotic lesion [13]. (Source: Stenvinkel et al 2003)

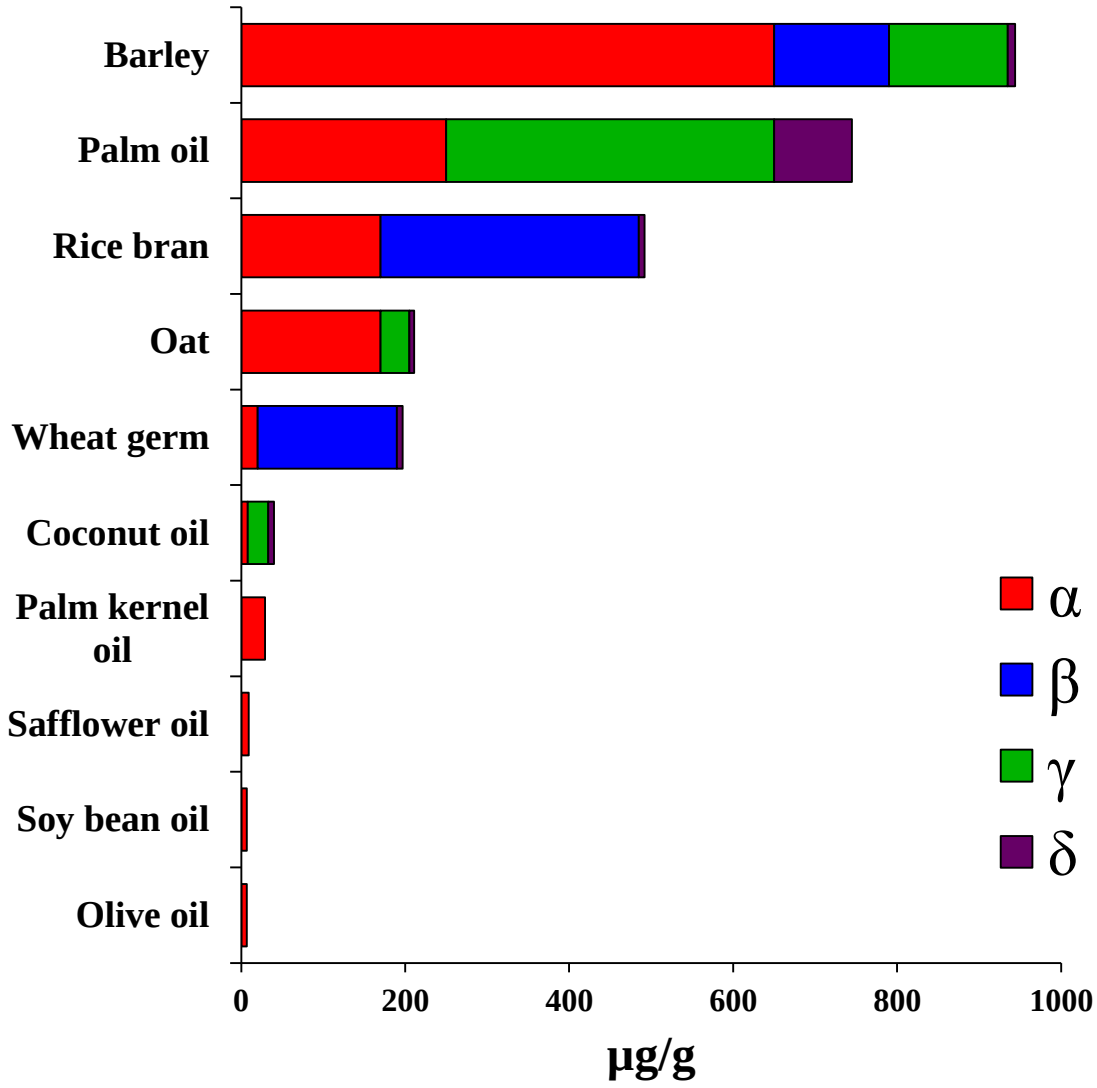
Figure 1-3. Vitamin E Tocotrienol and Tocopherol chemical formulas



(Source: Aggarwal et al 2010)

Depicts the eight (8) known forms of vitamin E – isomers, in order α-, β-, δ-, γ- tocotrienols (**TT**) vs. tocopherols (**TP**) differ based on the number of methyl group and chromanol ring (15-carbon tail). **TP** are also different from **TT** due to the present of saturated chain rather than unsaturated chain. **TT** which possess an isoprenoid side chain and have the presence of three trans-double bond in the tail distinguish it against **TP** [26].

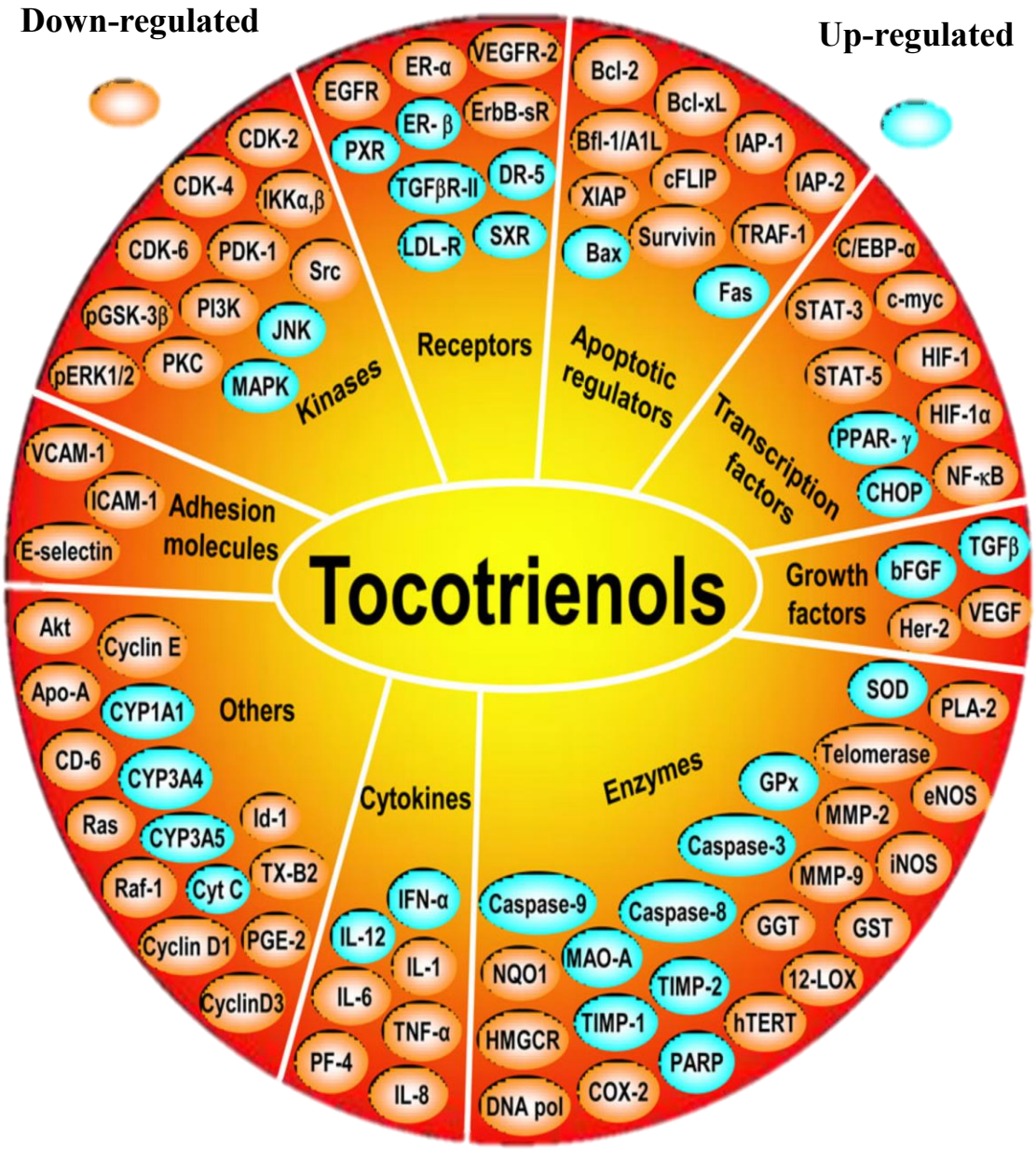
Figure 1-4. Natural dietary source of Tocotrienols



(Source: Aggarwal et al. 2003)

The figure shows the natural dietary sources of tocotrienol (TT), expressed in µg per gram. TT is abundant in palm oil, rice bran, barley, oat and wheat germ, while minimal contained in peanut oil, safflower, soybean, cocoa butter and olive oil [26].

Figure 1-5. Tocotrienol antioxidant/anti-inflammatory properties, effect in biomarkers



(Source: Aggarwal et al. 2003)

Figure depicts the molecular targets of Tocotrienols as an anti-inflammatory.

CHAPTER 2

MATERIALS AND METHODS

Study Design

The general aim of the study was to investigate the effects of supplementing vitamin E- tocotrienols on hemodialysis population, and after analyze any changes induced by this lipid altering, antioxidant, and anti-inflammatory agent, on the patients' metabolites profile. The study was double-blinded, randomized, parallel, placebo-controlled design trial, of 81 adult's patients undergoing chronic hemodialysis at Great Lake Dialysis Clinic, Detroit MI, where patients routinely received hemodialysis treatments 3 days a week. The protocol for this clinical trial was approved by the Human Investigation Committee of Great Lake Dialysis Clinic, (case: IRB #00007308) and Wayne State University (case: IRB #067411A).

Subject Selection

For this clinical trial intervention there were initially screened 118 patients. From this batch of patients, 37 were excluded due to the fact they did not meet the inclusion criteria (n=30), and (n=7) patients declined to participate. Thereafter 81 of these hemodialysis patients were randomly divided into two different groups before the intervention began; a placebo group (n=37) and intervention group TRF (n=40) **Figure 3-1**. The final number of participants were randomized into blocks in order to keep the size of treatment groups similar, and block size randomization selection was utilized in order to reduce any bias (Saghei 2004). In metabolomics analysis patients pool selected was (n=15) ESRD patients, of which (n=7) samples came from placebo group and (n=8) came

from TRF group. For this metabolomic analysis patients which utilized statin medication were excluded and only baseline and week 12 plasma samples were tested.

Nutritional Intervention

In order to provide a controlled environment to administer the supplement, gel capsules were given to both groups' placebo and TRF; on *dialysis* and *non-dialysis* days. During dialysis day capsules were oral consumed by the patients at the clinic where they were monitored by researchers in order to observe patients compliance in taking the capsules. For non-dialysis days capsules were provided for subjects to consume at home.

The placebo group supplement contents, consisted of wheat germ oil with a mixture of 0.24 mg of tocotrienols and 0.44 mg of tocopherols per capsule. The intervention group received tocotrienol-rich fraction (TRF) capsule consisted of palm fruit oil, containing a mixture of 90 mg of tocotrienols and 20 mg of tocopherols, for a total 110 mg of vitamin E per capsule. A summary of the TRF and placebo capsules composition that the participants were provided with, for consumption is as follows; both TRF and placebo capsules, the oil mixtures were composed of Tocopherol (TP) and Tocotrienol (TT) vitamin E which was measured in (mg). Each TRF capsule was composed of (20.0 mg) of α-TP and no (β, γ, δ)-TP into the mixture, than other main ingredient were comprised of (30.18 mg) of α-TT, (5.30 mg) of β-TT, (41.66 mg) of δ-TT, (12.86 mg) of γ-TT for an overall 90 mg of TT from palm fruit oil. The composition of placebo capsule was made of (0.29 mg) of α-TP, (0.04 mg) of β-TP, no- δ-TP, (0.11 mg) of γ-TP, and into the mixture (0.12 mg) of α-TT, (0.06 mg) of β-TT, no- δ-TT, (0.06 mg) of γ-TT, for an overall 0.68 mg. Therefore the placebo control group capsule had 0.24 mg of TT and 0.44 mg of TP derived from

wheat germ oil. The established daily dose supplemented orally for patients was: 2 x 110 mg/day as provided by (Carotino, Johor Darul Takzim, Malaysia) for a total of 220 mg/day, for 16 weeks [35].

Blood Samples Collection and Handling

Blood samples were collected from the patients at the start of the dialysis session into vacutainer tubes containing EDTA or heparin and transported to Wayne State University within 2 hours of collection. Additional blood was collected into separate tubes without anticoagulant for standard renal profiles measurements which were sent to an external laboratory (Ser., Alb, Hb, Kt/V) (Ascend Clinical Laboratory Services, Redwood City, CA, USA). The tubes which contained EDTA were designed for the analyses of lipid profiles and NF_KB, whereas lithium heparin tubes were for the analysis of oxidative status and inflammatory markers. The plasma processing and separation was conducted by centrifugation at 2800 rpm for 20 minutes at 4°C (GS-6KR Centrifuge, Beckman-Coulter). The aliquots of plasma were then stored at -80°C. In the case of the blood samples which were used to analyze nuclear factor kappa B (NF_KB) were processed immediately in order to obtain mononuclear cell extract using Ficoll-Paque method.

Anthropometry Measurements

Anthropometric measurements were carried out by estimating dry weight and height at the baseline and the end of the study. The height was measured to the nearest 0.1 cm using whereas the body weight was measured at the nearest 0.1 kg, after each dialysis session. BMI was calculated from the measurements of weights over height using

Quetelet's Index [36] and the data was compared with WHO classification database charts [37].

Biochemistry Assay, (Lipids Profiles)

For biochemical analysis this clinical study focused on measuring renal profiles, lipid profiles, and inflammatory markers.

1) *Renal profile* was determined based on standard measures: Serum albumin (ser. Alb.), hemoglobin (Hb), and (Kt/V). This analysis was performed by external laboratories.
2) *Lipid profiles*: Total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), and Cholesteryl ester transfer protein (CETP) analysis were conducted using enzymatic assays.
3) For *inflammatory markers*, the conducted measurements were: C-reactive protein (CRP) and nuclear factor kappa B (NFκB).

Lipid Profiles

For lipoprotein profile, the study measured TC and TAG levels using commercially available enzymatic kits (Pointe Scientific, Canton, MI) and values were expressed as mg/dL for all samples [35].

Measurements for HDL-C were conducted in the supernatant fraction following precipitation of ApoB contacting lipoprotein from. LDL-C was calculated using the Friedewald's equation by difference [35, 38]. CETP activity in the plasma was measured using fluorometric assay kit. In this case the intensity was measured using a fluorometer at 465nm wavelength quantified and expressed as pmoles/ μL plasma/hour [35] per protocol.

Inflammatory Markers

To measure C-reactive protein (CRP) activity commercial kits were used (Cayman Chemical, Ann Arbor, MI). In this case the plasma samples were diluted in the assay 1:15,000 and 100 μL of each sample placed into 96-wells micro-plate pre-coated with a monoclonal antibody specific to human CRP and then incubated. The well-plates were rinse 4 times with wash buffer before adding HRP-labeled CRP monoclonal antibody. Two antibody were added to form a sandwich by binding to different location in the CRP molecule samples. Lastly CRP concentration in the plasma samples were measured at 450nm wavelength after adding chromogenic substrate TMB (tetramethylbenzidine) which formed a yellow color. In this case standard curve was calculated using a known concentration of CRP and the data was expressed in mg/L.

Measurements for the NFκB activity in the plasma was conducted in three different steps. The first step was 1) isolation of mononuclear cells from blood, 2) extraction of nuclear and cytoplasmic extract, and 3) measuring of NFκB expression and concentration in the nuclear extract. NFκB expression was measured using kits purchased by TransAM NFκB (Active Motif, Carlsbad, CA, USA) as per the manufactures protocol.

Metabolomics Analysis:

Blood Samples Collection and Preparation for NMR Analysis

NMR analysis samples were randomized and obtained from the original sample pool of subjects than selected (n=8) TRF samples and (n=7) placebo. First retrieved from -80°C and allowed to thaw and equilibrated at room temperature. After the thawing process was over the samples were further centrifuged at 10,000 rpm for roughly 2 minutes, in order to remove any solid debris. Than 420μL of plasma supernatant were aliquot into pre-

assembled centrifugal filter and filtrate collecting tube units by Amicon Ultra with filter parameters of 0.5mL of 3kDa unit (Sigma-Aldrich, St. Louis, MO, USA) on which cases the filter units were washed three times prior with deionized water (450 μL deionized water, and spun for 14,000 rpm for 5 minutes) as protocol per say. Plasma was aliquot into each of the filtered unit tubes, than samples were centrifuged for 30 minutes at 14,000 rpm at room temperature. The filtrates from previews step were transferred into new microtubes at which they were prepared for dilution with deuterium oxide (D_2O) to equilibrate them into a 1:1 ratio (300μL filtrate + 300μL D_2O). The final NMR working solution, was composed of (**300μL filtrate + 300μL D_2O**), plus additionally added **5mmol/L** 3-(trimehylsilyl)-1 propanesulfonic acid (**TMS**) and **10mmol/L** Imidazole to bring the final sample solutions into a dilute made out of 9:1 ratio. These final NMR samples are then transferred into 5mm NMR tubes (8inches long) for analysis.

Proton ^{1}H NMR acquisition and Spectral Processing

To acquire ^{1}H NMR spectra, a 600MHz Agilent nuclear magnetic resonance spectrometer was used at the temperature of 27°C to read the samples. This specific custom made design protocol, utilized to measure free induction decay (FIDs) focused in collecting data at 32K data-points, for a total of 64 scans, with the spectrum width of 10ppm, and the acquisition time of 4 seconds. The water signal in this study were suppressed and this was accomplished by pre-saturation and set the flip angle reading to 90° degrees.

After gathering the NMR FIDs data, each file was processed using ACD software version 12.0 (Advance Chemistry Development, In., Toronto, ON, Canada) by using the stacking technique, were 64 scanned data were compiled followed by: 1) Fourier transformation, 2) auto-phasing, and 3) auto-baseline correction protocols. The full spectra

for each measured sample were divided into 1000 equal bins using intelligent binning option. Than after the spectra were digitized into a table of common integral containing a non-negative value, the data was finally exported as text files, and transferred in SIMCA-P to perform multivariate data analysis.

Multivariate Data Analysis

To perform multivariate data analysis SIMCA-P 13.05 (Umetrics, Umea, Sweden) was used. In SIMCA each row represent a case (i.e. a patient plasma) whereas each column represents a variable (i.e. the 1000 binned spectras). The NMR data pertaining to metabolites was statistically analyzed using three types of mathematical algorithms: 1) principle component analysis (**PCA**), 2) partial least square (**PLS**) and 3) partial least square discriminant analysis (**PLS-DA**).

PCA was utilized first because is the simplest model which analyses values and the best way to get the overview picture of the model before exploring into further depth. The informations in this model resides in the correlation structure of data, were mathematical principle explores projection of lower dimensionality designed to display the systematic variation in data matrix-X in order to show related observations and deviations similarities in the data set, via visual display from a score or loading plots. A PCA score plot is designed to explain a visual summary (i.e. pattern, trends, and clusters,) of the observation and information regarding the similarities and differences in the data (i.e. control group vs. intervention group). The visual depiction corresponding to the loading plot is used to explain and provide information regarding the variables (part of the spectrum i.e. metabolite which may cause a difference), that maybe be responsible for difference and similarities observed in the score plot [39].

The other algorithms used for this intervention were PLS and PLS-DA which are considered to be supervised pattern recognition analysis which is described as discriminant analysis which looks at differences between groups in the study [39]. PLS is a regression extension of PCA in which the Y variable(s) are added to connect with the information provided by X variable(s). Therefore in PLS analysis the conducted analysis depicts two matrices X (e.g. chemical descriptors) and Y (e.g. biological responses) and has two objectives which deals with the relationship between X and Y [40]. Additionally PLS-DA tends to identify the models which separates the classes of observation based from X variable(s) to the hypothetical Y variable(s). In all the models the X variable(s) were previously mean-centered and Pareto-scaled as the basis for block-scaling before performing multivariate data analysis [41].

Metabolites Identification and Quantification

The metabolites were identified and quantified using Chenomx NMR 7.1 (Edmonton, Alberta, Canada), in which FIDs files were first processed to adjust for pH, assign chemical shift indicator, than remove line shape distortion by auto-phasing, and lastly auto-spline the baseline correction then reference de-convolution. The spectra in text form were exported into Chenomx profiler for identification and quantification of the metabolites for which there are 313 known metabolites in this database specific for a 600 MHz NMR. Each corresponding peak pertaining to a metabolite, were matched to the existing database and the area under the peaks were used as standard which indicated the literature concentration and manually each metabolite was adjusted to matching the database spectra.

Statistical Analysis

Data results from experiments were keyed into Microsoft Excel and organized accordantly, thereafter these data were transferred into SPSS to further conduct detailed analysis. Statistical analysis was carried out using SPSS (version 22 IBM,) and results were presented in forms of mean ± SEM (mean ± standard error mean) on the tables and the figures. Data that were not normally distributed were presented as median ± IQR (median ± Interquartile ranges). Categorical data were presented as absolute numbers and percentages. Independent t-test analysis was utilized when necessary to compare values which applied to normally distributed data, to check for differences in means between two groups. Paired t-test analysis was used to check for differences between time points, and chi-square test was used to test the differences in categorical data.

CHAPTER 3

RESULTS

Characteristics of study population

Figure 3-1 describes TRF study selection and grouping of patients, which were allocation for the duration of this clinical intervention as well as the inclusion and exclusion factor values. A total of 81 ESRD patients were randomized into two different groups placebo (n=37) and TRF (n=40). Both groups were provided with placebo or TRF specially designed capsules for 16 weeks. ESRD patients were further divided into four subgroups for further in-depth analysis of the lipid profiles and biomarkers.

Table 3-1 shows the clinical and demographic characteristics of the study population. No significant differences were observed in all demographics and or clinical variable measurements when comparing the data from baseline to week 16. The population was homogenously comprised of African American patients with a semi-equal distribution in genders of 43 males and 38 females out of 81 overall participants.

Table 3-2. Depicts the effect of TRF supplementation on plasma lipids in subset of subjects that were taking statin drugs and those who were not. All groups were crossed analyzed against each other in order to explore any differences in the lipid profile levels (TAG, TC, LDL-C and HDL-C) in which case values are presented as mean ± SEM. After various examinations, significant values between groups were reported by using superscripts, in which case different superscripts indicate significant differences ($p<0.05$) between groups within a row. The four groups were compared against each other at allocated times, baseline, week 8, week 12 and week 16. Analysis was conducted using independent t-test. Results showed decreases in TAG levels in plasma over time for TRF

Non-Statin and TRF Statin groups. Significant decreases were also observed in weeks 8 and 16 among Placebo Non-Statin and (TRF Non-Statin/Placebo Statin) groups when compared. On the other had Placebo Non-Statin and Placebo Statin did not record any decreases of significance in TAG levels. During TC analysis, TRF Non-Statin group saw a decrease in levels from baseline to 16 weeks, whereas TRF Statin observed a decrease upon week 12 and not beyond. A comparison between the two groups did record a significant decrease in TC levels at week 16 in which case favored TRF Non-Statin over TRF Statin. There was a marginal decreasing trends of TC level among Placebo Non-Statin/Statin groups up until week 12. LDL-C also observed a decrease in its levels over time when looking at TRF Non-Statin and TRF Statin groups. Furth more at weeks 12 and 16 analysis showed significant decrease among the two groups. Placebo Statin showed a marginal change in LDL-C, whereas Non-Statin displayed inconclusive results. HDL-C analysis, displayed a significant increases in both groups TRF Statin/Non-Statin over time. Incremental increase was recorded in Placebo Statin/Non-Statin groups which were of no significance. A comparison between groups showed significance difference between Placebo Non-Statin and TRF Non-Statin, at which point placebo had lower HDL-C levels at baseline, but after this point levels of HDL-C increase in both groups as time went on; in the end favoring the TRF Non-Statin group with higher HDL-C levels.

Table 3-3. It represents eleven metabolites which were discovered to have changed significantly ($p<0.05$) on ESRD plasma samples in TRF group when compared to the placebo group. The metabolites were quantified and identified using Chenomx, whereas statistical analysis was conducted using SPSS., in which case values are represented as

mean ± SEM. Eight out of eleven metabolites showed a significant decrease due to the TRF intervention and three out eleven metabolites showed a significant increase.

Figure 3-2. Explores changes in CETP levels per hour between TRF and placebo groups. Values are presented as mean ± SEM, and the units of measurement are pmol/µl. Changes of CETP activity in the plasma were analyzed and compared among the four sub-groups, using independent t-test at baseline, week 12 and week 16. At week 16 a significant decrease ($p<0.0001$) in CETP was observed in TRF Non-Statin and TRF Statin groups when compared against Placebo Non-Statin. Likewise Placebo Statin group showed a marginal decrease in CETP level when compared to Placebo Non-Statin.

Figure 3-3. It depicts the differences in CRP levels between the TRF groups and placebo groups. Values are presented as mean ± SEM and the measured units used were mg/L at baseline, week 12, week 16, and week 8 was not measured. Lower CRP levels were observed at baseline for statin TRF and non-statin placebo when compared in parallel to changes in activity for Placebo Statin and TRF Non-Statin groups. Than on week 12 CRP levels increase significantly for TRF Statin, and Placebo Non-statin. At week 16 no significant changes in CRP levels were observed between the groups. Different superscripts are indicates of significance ($p<0.05$) change in the CRP activity levels.

Figure 3-4. Depicts NF_KB activity between TRF and placebo groups. Values are presented as mean ± SEM and the measured units used was pg/mL at baseline, and week 12, no measurements were conducted at week 8 or 16. A decreased was observed at baseline between TRF Non-Statin and TRF Statin, and these changes were reported with

different superscripts indicating significance ($p<0.05$). Week 12 showed no change in NFκB activity between the groups.

Figure 3-5. Is the depiction of the changes in TAG levels over time among statin and non-statin groups. Values are presented as mean ± SEM based on independent t-test analysis. A decrease of TAG levels was recorded within groups TRF Statin and TRF S Non-Statin at week 12 ($p<0.05$), but only a marginal decrease trend was observed at week 8 and 16 for the following groups. No significant difference were observed for Placebo Statin and Placebo Non-Statin groups at either weeks 8,12, or 16 even though there are marginal decrease in TAG levels within the study population.

Figure 3-6. It is the change in time of HDL-C levels among TRF and placebo groups. Values are presented as mean ± SEM, were different letters indicate significant changes over time per group ($p<0.05$). All groups during week 8 saw no changes in HDL-C levels but a gradual increases in this lipoprotein was observed at week 12 and 16 on all the groups. Significant differences were seen between TRF Statin and TRF Non-Statin at both times week 12 and 16 when compared to baseline results.

Figure 3-7. Shows a PCA score plot for the NMR spectra using plasma samples on ESRD patients at baseline, in order to observe grouping behavior. The figure overviews the metabolomic profiles of patients who were grouped under placebo and TRF groups and observes similarities or dissimilarities on metabolite profiles found in plasma. This PCA showed no correlation between the two groups. The inside of the eclipse represents 95^{th} percentile of confidence interval and the outside of the eclipse is considered an outlier.

Figure 3-8. Depicts the PCA score plot for NMR spectra using plasma samples on ESRD patients measured at week 12. This PCA model shows a moderate separation between the two groups, based on the cluster formation in relation to the eclipse graph. The inside of the eclipse represents 95^{th} percentile of confidence interval and the outside of the eclipse is considered an outlier and this model shows one TRF outlier in the PCA model.

Figure 3-9. PLS-DA score plot from NMR spectra using plasma samples from ESRD patients measured at week 12. This model show a strong correlation and clear separation between the groups, than previews PCA score plot. The separation of two groups and the cluster formation are observed by samples being located on different planar sides of the eclipse.

Figure 3-10. The PLS score plot shows the correlation of metabolomic profiles and plasma lipids profiles of TC, TAG, and HDL-C measured at week 12. It depicts the correlation between metabolomic profiles on the (x-axis) and plasma lipid profiles (TC, TAG, and HDL-C) on (y-axis) between the two groups. The model shows a moderate correlation (R^2 = 0.597) between the metabolomic profiles when compared the lipid profiles, and a clear separation between the two groups when looking at the samples (TRF and Placebo) location on the graph's plains of symmetry.

Figure 3-11. PLS score plot correlates plasma metabolite profiles to CRP and IL-6 levels. It shows a correlation between metabolomic profiles on the (x-axis) and inflammatory markers on (y-axis) between the two groups at week 12. The model shows a small moderate correlation (R^2 = 0.38) between the two groups and the variables. A clear

separation between the two groups is observed and clusters are formed from the two groups which are located at opposite planes of graph regions.

Figure 3-12. PLS score plot, correlation of metabolomic profiles in the plasma and serum albumin at week 12. It depicts the correlation between the plasma metabolomic profiles on the (x-axis) and the serum albumin (marker of nutritional status in ESRD patients) (y-axis) at week 12. The results indicate a strong correlation between the two groups with a ($R^2 = 0.645$) values which is significant.

Figure 3-13. Is a PLS model correlating antioxidant activity (TAP and MDA) - (y-axis) compared to metabolomic profiles in the plasma located on the (x-axis) at week 12. The results indicate a small moderate correlation between the two groups which is represented by ($R^2 = 0.41$) values.

Table 3-1. The table depicts baseline clinical and demographic characters of the study population.

	Placebo (n=40)	TRF (n=41)	P value
Demographics			
Age (yrs)	58±13	59±12	0.565
Age > 65 yrs (n, %)	12 (30)	14 (34)	0.689
Ethnicity			
African American (n, %)	40 (100)	40 (97.5)	-
Gender (males, %)	23 (57.5)	20 (48.8)	0.432
Smoking cigarettes (n, %)	10 (25.0)	10 (24.3)	
Clinical			
Diabetes Mellitus (n, %)	26 (65)	25 (61)	0.708
Hepatitis C (n, %)	5 (12.5)	5 (12.2)	0.967
Time on dialysis (months)	37±18	34±19	0.446
BMI (kg/m^2)	28.7±8.2	30.3±8.1	0.768
Systolic B/P (mmHg)	150±24	153±23	0.651
Diastolic B/P (mmHg)	81±15	83±14	0.506
Kt/v	1.48±0.26	1.45±0.20	0.516
Statins (n, %)	11 (27.5)	17 (41.4)	0.124
Aspirin (n, %)	22 (55.0)	25 (61.0)	0.297
Anti-hypertensive, one or more type (n,%)	35 (87.5)	34 (82.9)	0.562
Vascular access			0.732
Arteriovenous fistula (n, %)	18 (45)	15 (36.6)	
Arteriovenous graft (n, %)	13 (32.5)	16 (39)	
Venous catheter (n, %)	9 (22.5)	10 (24.4)	

BMI=Body Mass Index; **B/P**=Blood Pressure; **Kt/v**=Index of Dialysis adequacy

The data is reported as mean ±SD. P-value (< 0.05) served as a comparison between placebo and TRF groups and no significant difference was observed [35].

Table 3-2. Effects of Tocotrienol supplementation on plasma lipids in patients also prescribed statin drugs.

Lipid Profiles		TRF Non-Statin (n=23)	TRF Statin (n=17)	Placebo Non-Statin (n=26)	Placebo Statin (n=11)
TAG	*Baseline*	121±17	157±43	116±13	154±24
(mg/dL)	*Week 8*	129±17 ^{a}	135±23 ^{ab}	98±9 ^{b}	154±24 ^{a}
	Week 12	101±8	119±13	103±12	112±17
	Week 16	80±7 ^{b}	98±11 ^{ab}	104±11 ^{a}	126±16 ^{ab}
TC	*Baseline*	184±8	187±14	173±8	186±15
(mg/dL)	*Week 8*	159±5	159±11	150±7	154±11
	Week 12	145±7	145±12	140±6	126±14
	Week 16	140±8 ^{b}	154±13 ^{a}	150±8 ^{ab}	141±13 ^{ab}
LDL-C	*Baseline*	115±8	113±15	108±7	110±14
(mg/dL)	*Week 8*	80±6	81±10	81±6	71±10
	Week 12	67±7 ^{a}	54±11 ^{b}	73±6 ^{ab}	47±12 ^{ab}
	Week 16	65±8 ^{b}	74±13 ^{a}	77±7 ^{ab}	60±12 ^{ab}
HDL-C	*Baseline*	45±3 ^{a}	42±4 ^{ab}	42±2 ^{b}	45±3 ^{ab}
(mg/dL)	*Week 8*	53±4	52±4	49±2	52±5
	Week 12	59±3	68±5	51±2	63±4
	Week 16	59±2.8	60±6	52±2	56±6

TC- Total cholesterol; HDL-C - High-density lipoprotein cholesterol; LDL-C - Low-density lipoprotein cholesterol; TAG - triglycerides

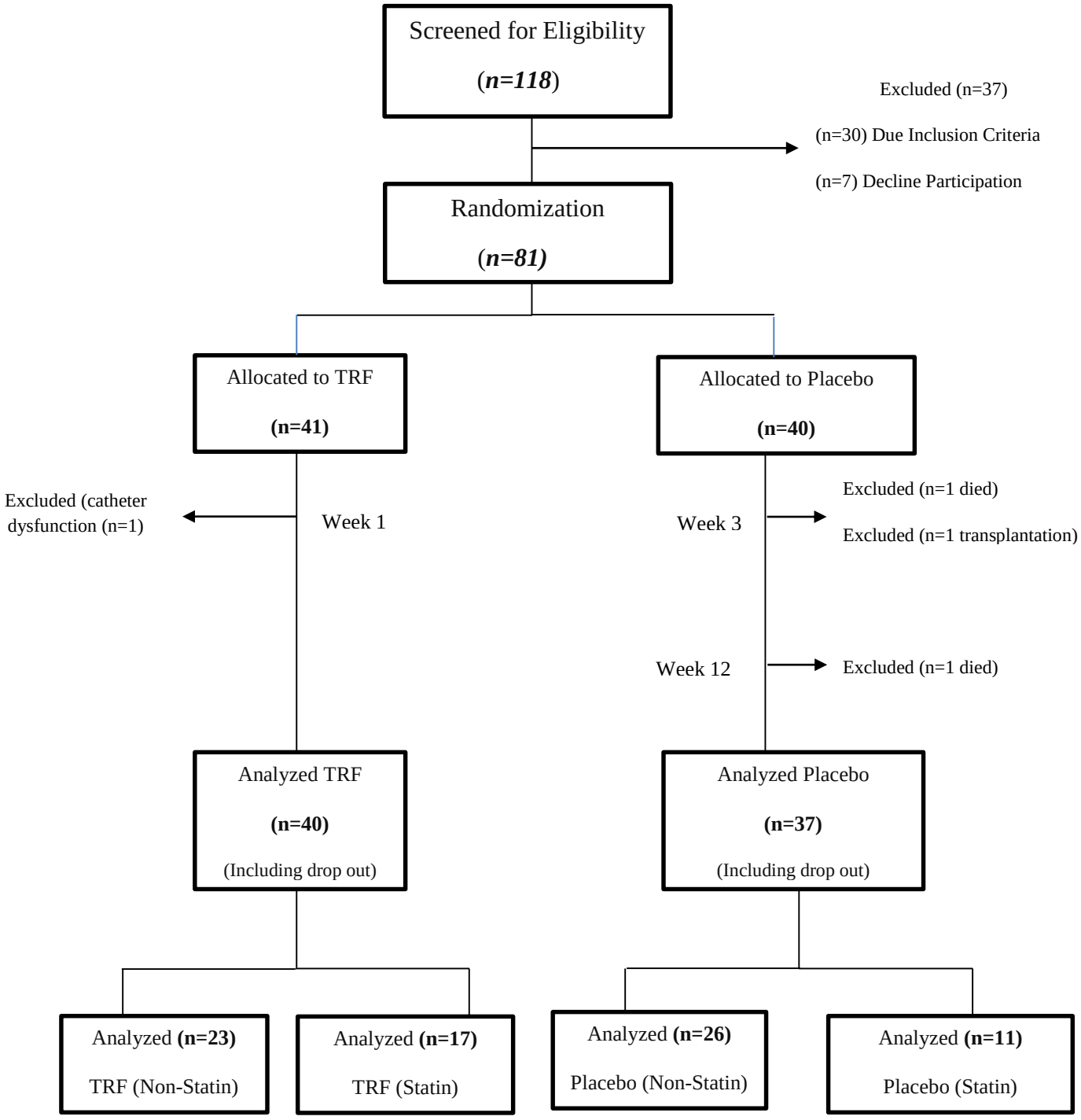
Values are presented as mean ± SEM. Different superscripts indicate significant difference ($p<0.05$) between groups within a row. The four groups were compared against each other at allocated times, baseline, week 8, week 12 and week 16. Analysis was conducted using independent t-test.

Metabolites of Change on ESRD Patients using Chenomx analysis peak (ppm)	Inc/Dec of TRF when compared to placebo	P-Value
10	Decrease	0.006
12	Decrease	0.021
34	Decrease	0.004
93	Decrease	0.034
97	Decrease	0.007
109	Increase	0.006
150	Decrease	0.017
192	Decrease	0.042
204	Increase	0.027
248	Decrease	0.038
259	Increase	0.045

Table 3-3. Metabolites of change on ESRD patients using Chenomx identification analysis when comparing TRF and placebo groups.

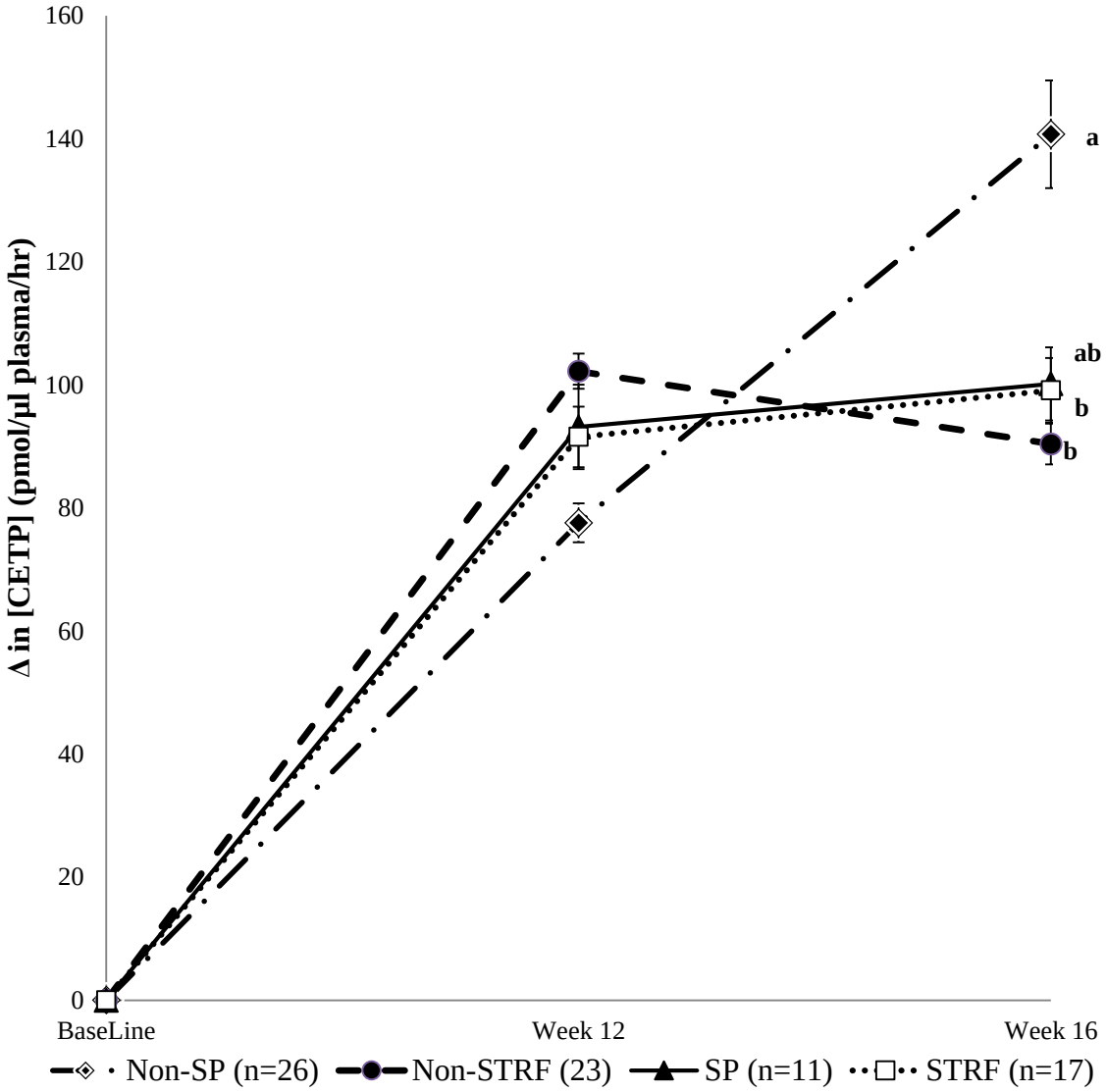
Note: Each sample number correlates to a peak (ppm) destination which has changed when analyzing NMR spectrums under Chenomx analysis. P-values are represented as mean ± SEM, and they indicates significant difference ($p<0.05$).

Figure 3-1. Depicts TRF study selection of patients' flow chart.



From (n=118) to (n=81) which were selected to be used for the clinical intervention. Patients were divided into two groups TRF (n=40) and Placebo (n=37). Subjects were further divided into four subgroups [TRF (Non-Statin) & TRF (Statin)] and [Placebo (Non-Statin) & Placebo (Statin)], in order to compare lipid changes.

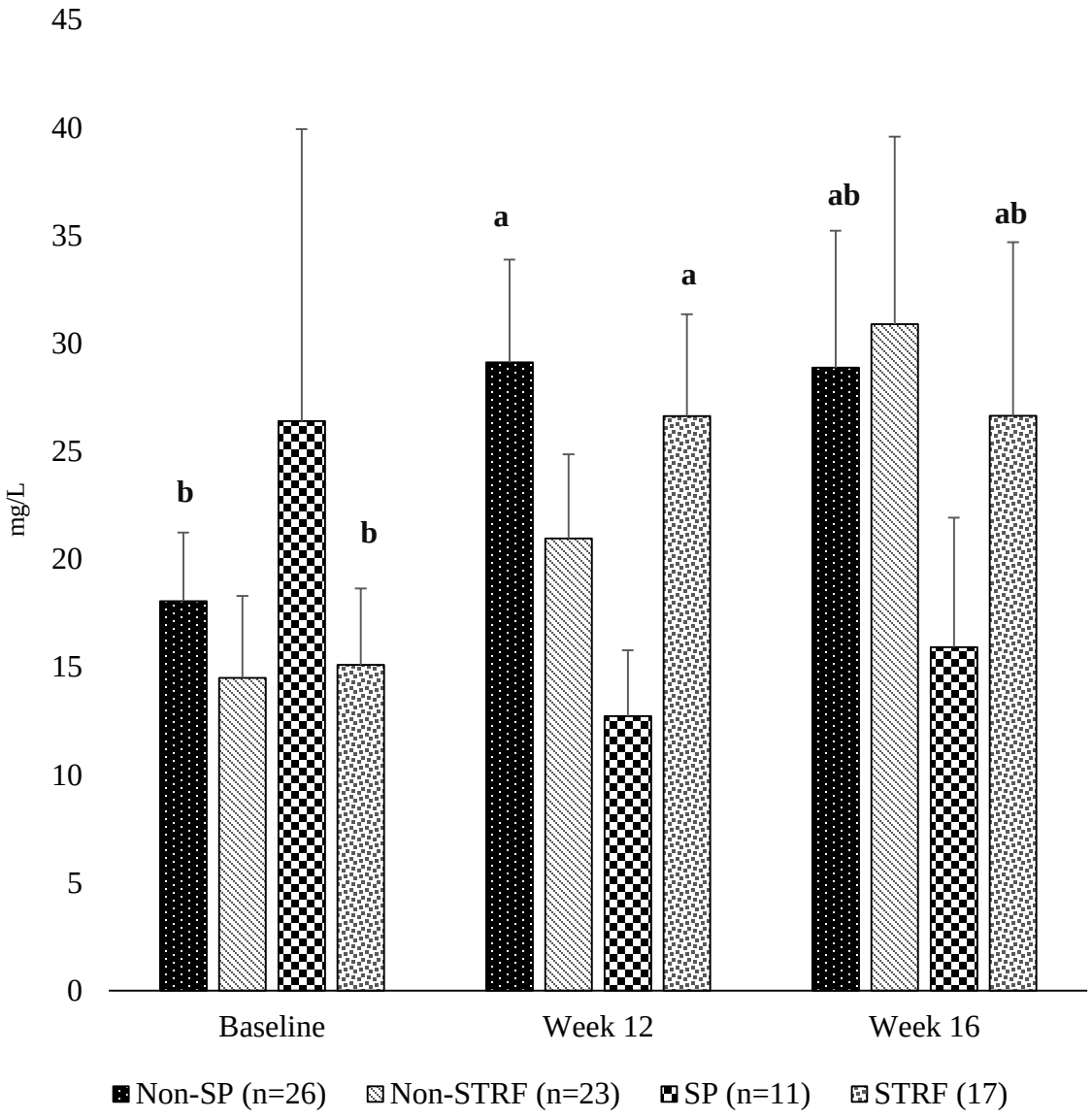
Figure 3-2. Comparison of CETP levels over time in TRF and Placebo groups.



SP-Statin Placebo, STRF-Statin TRF

Values are presented as mean ± SEM. Different superscripts indicate significant difference in CETP levels ($p<0.0001$). Non-Statin TRF and Statin TRF observed significant decreases when compared to Non-Statin Placebo. Whereas Statin Placebo showed a marginal decrease in conjunction to Non-Statin Placebo. Analysis was conducted using independent t-test, at allocated times, baseline, week 12 and week 16 and only difference was observed on week 16.

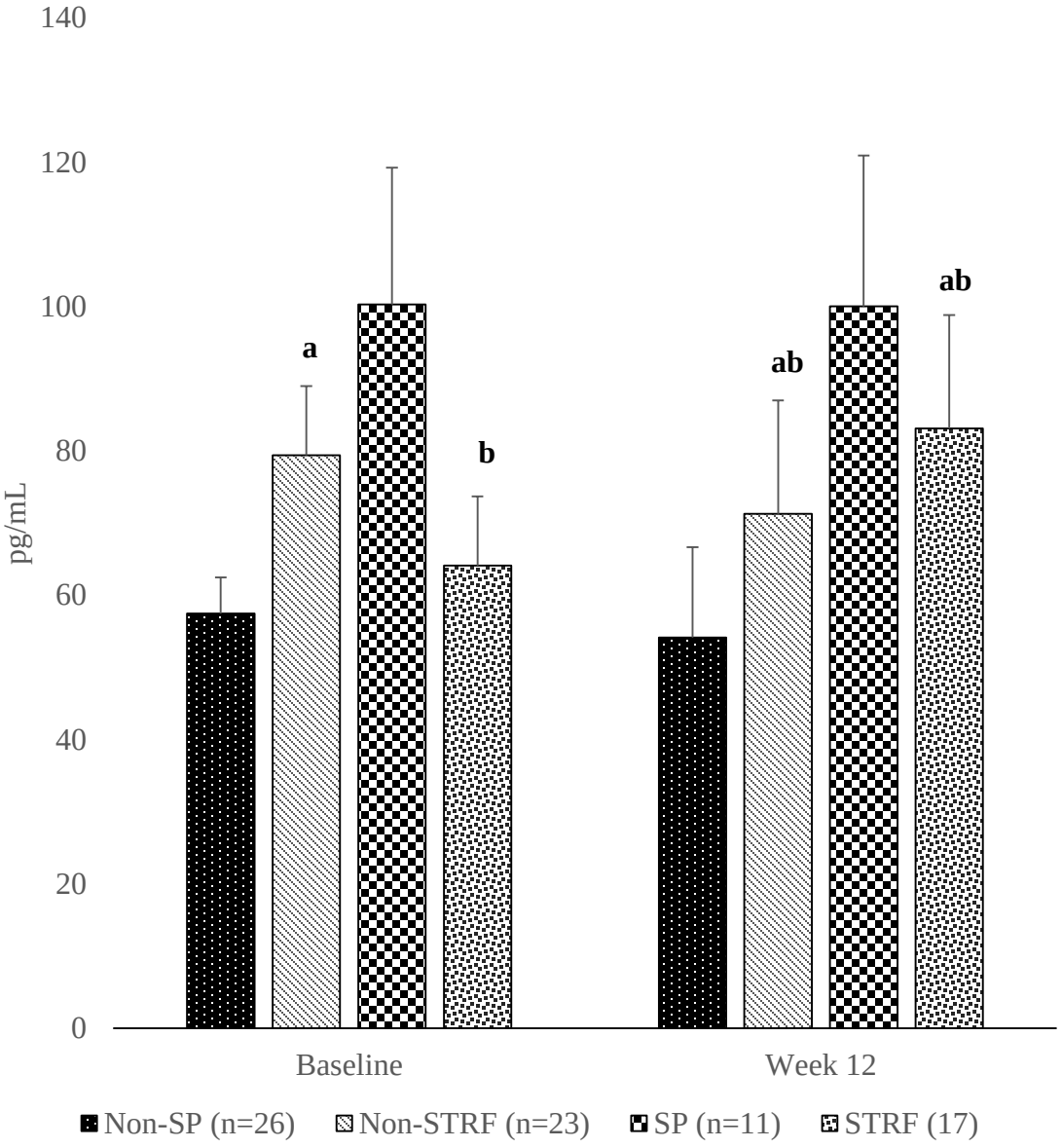
Figure 3-3. CRP comparison between TRF groups and Placebo groups



SP-Statin Placebo, STRF-Statin TRF

Values are presented as mean ± SEM. Different superscripts indicate significant difference ($p<0.05$) between groups.

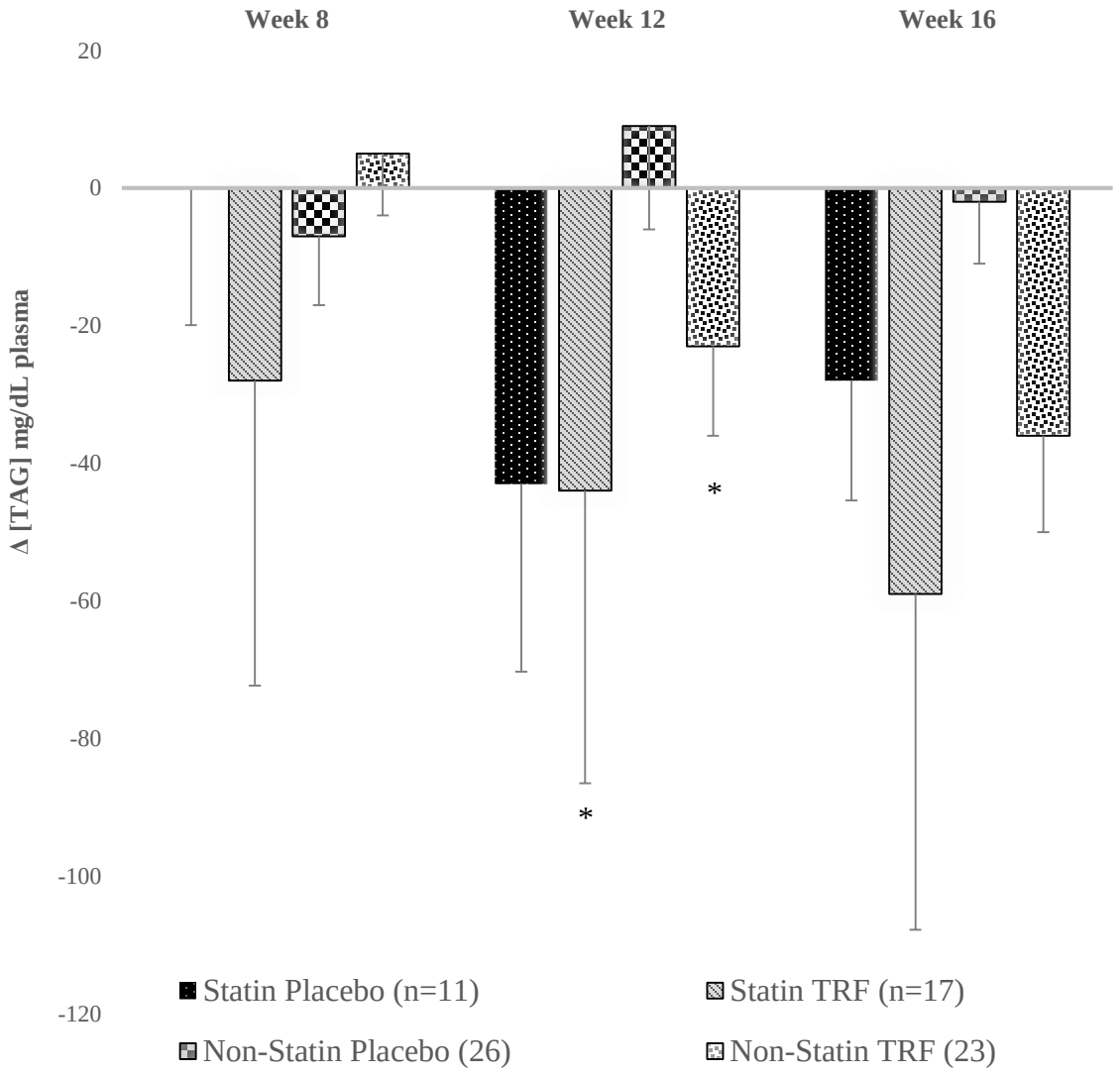
Figure 3-4. NFκB activity between TRF and Placebo groups.



SP-Statin Placebo, STRF-Statin TRF

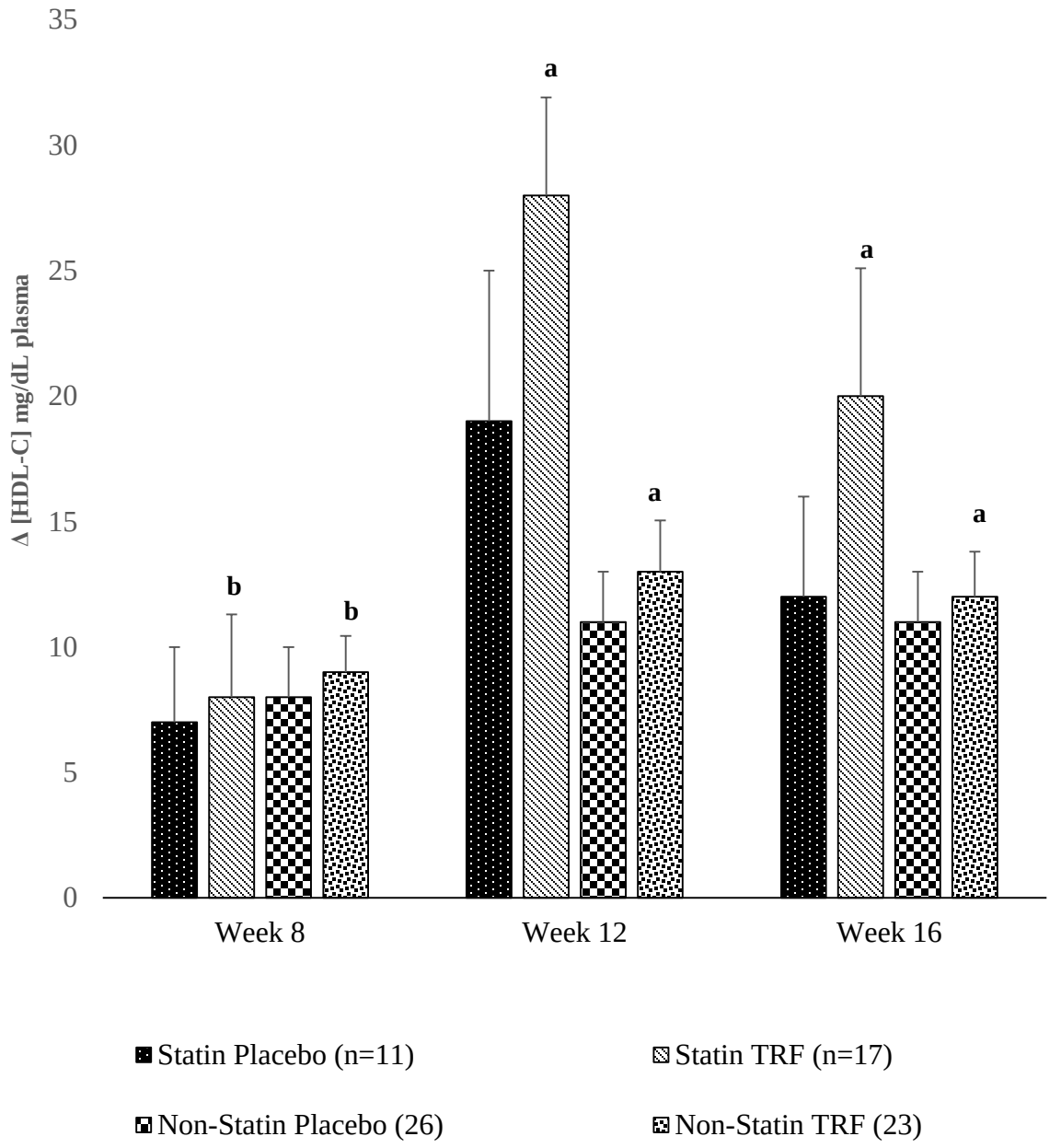
Values are presented as mean ± SEM. Different superscripts indicate significant difference ($p<0.05$) between groups. Statin placebo was compared to non-statin placebo, for the NFκB activity in the plasma, at allocated times, baseline and week 12. Analysis was conducted using independent t-test. Significance was observed at baseline a) $p=0.052$ and b) $p=0.051$ and at week 12 no significance was observed.

Figure 3-5. Change in TAG Levels among Statin and Non-Statin Groups



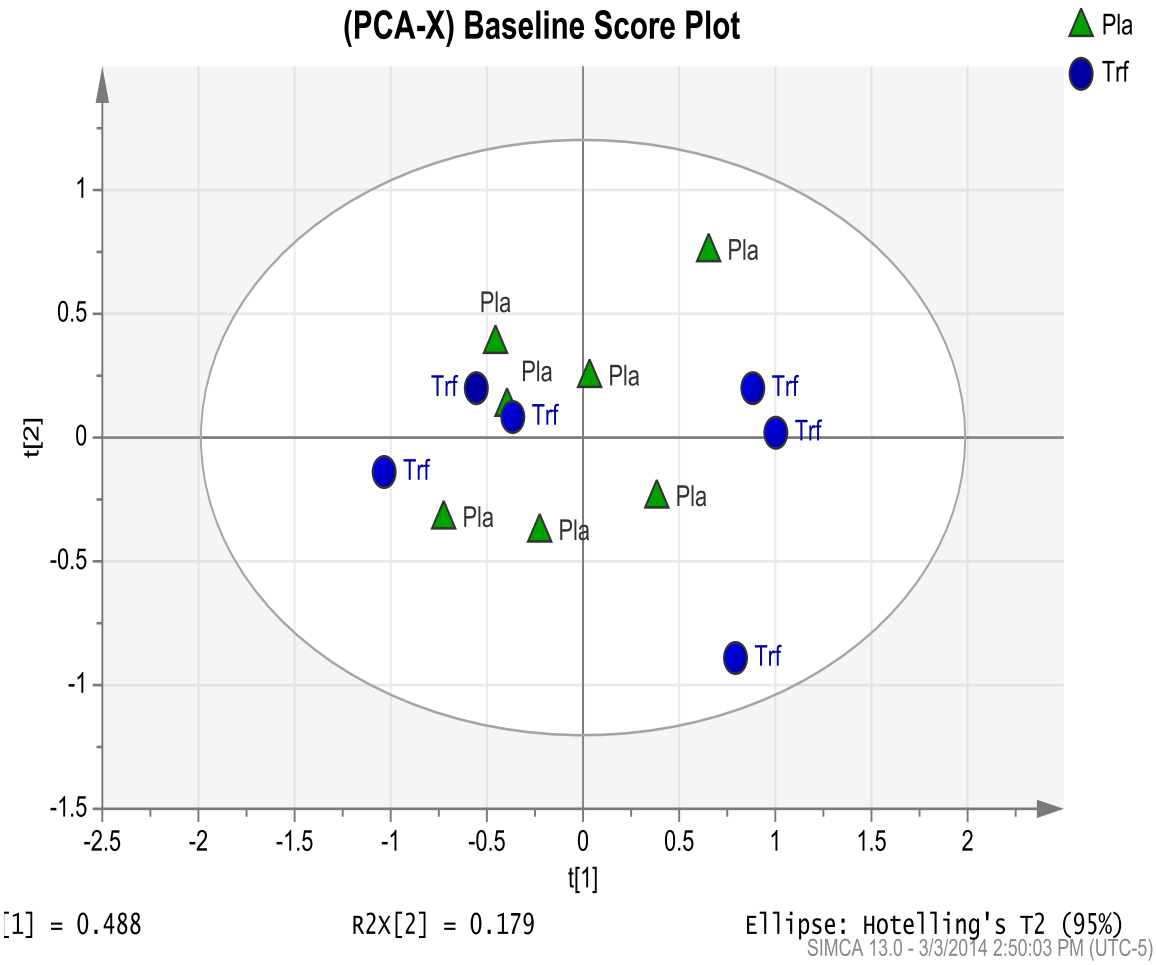
Values are presented as mean ± SEM. The graph depicts the decreasing TAG levels over the course of time, on all four groups. TAG data was calculated by different from baseline values. Symbol ***** indicates significant difference ($p<0.05$), based on independent t-test analysis.

Figure 3-6. Change in time of HDL-C levels among Statin and Non-Statin Groups



Values are presented as mean ± SEM. The graph depicts the increasing HDL-C levels over the course of time, on all four groups. HDL-C data was calculated by different from baseline values. Different letters indicate significant changes over time per group ($p<0.05$), based on independent t-test analysis.

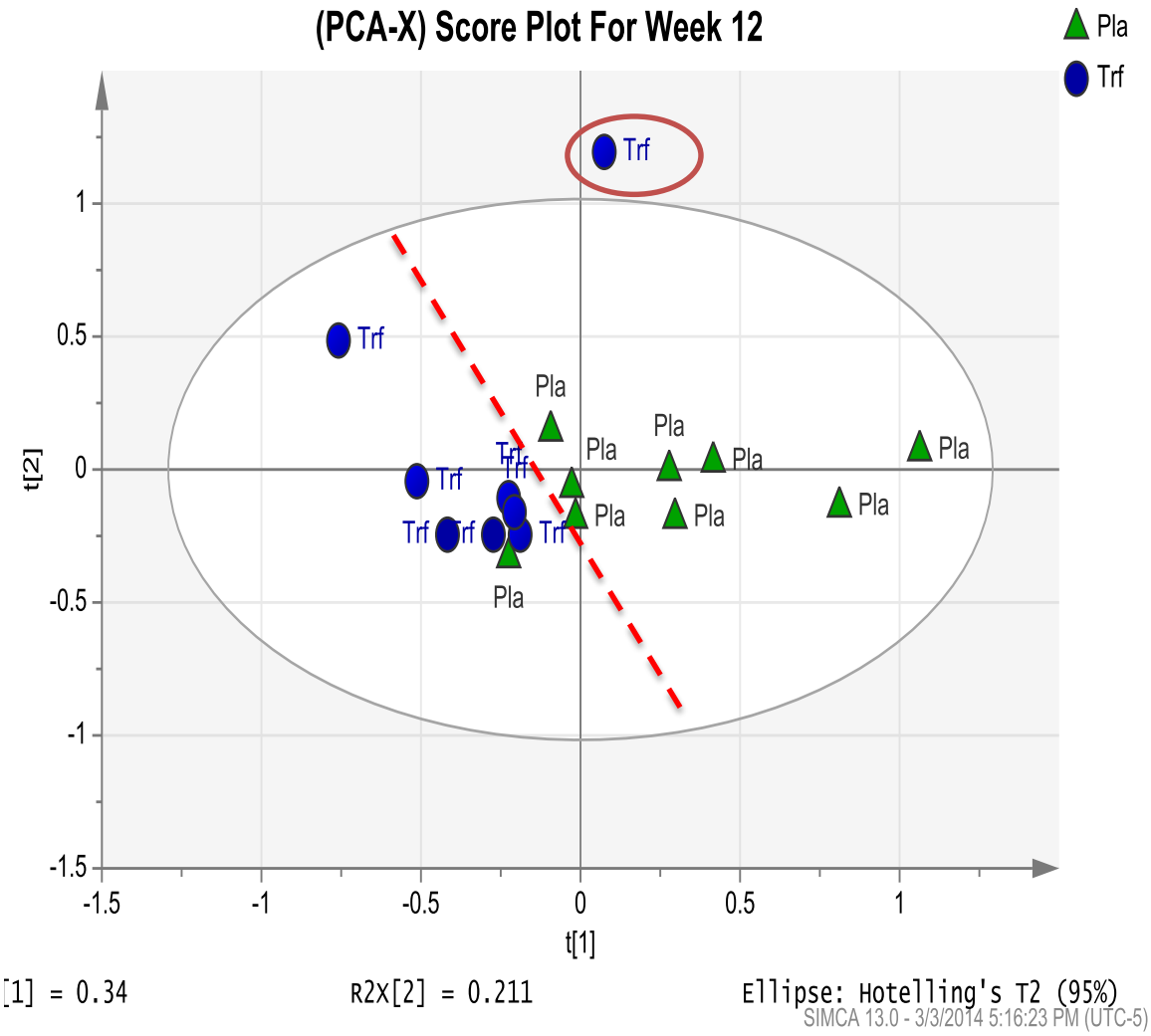
Figure 3-7. PCA score plot from NMR spectra using plasma samples from ESRD patients at baseline



PCA- principal component analysis; Pla-placebo; Trf- tocotrienol-rich fraction.

The figure is a depiction of PCA-X score plot at baseline, indicating the metabolomic profiles of ESRD patients under the placebo and TRF treatments. The inside of the eclipse represents 95th percentile of confidence interval and the outside of the eclipse is considered an outlier.

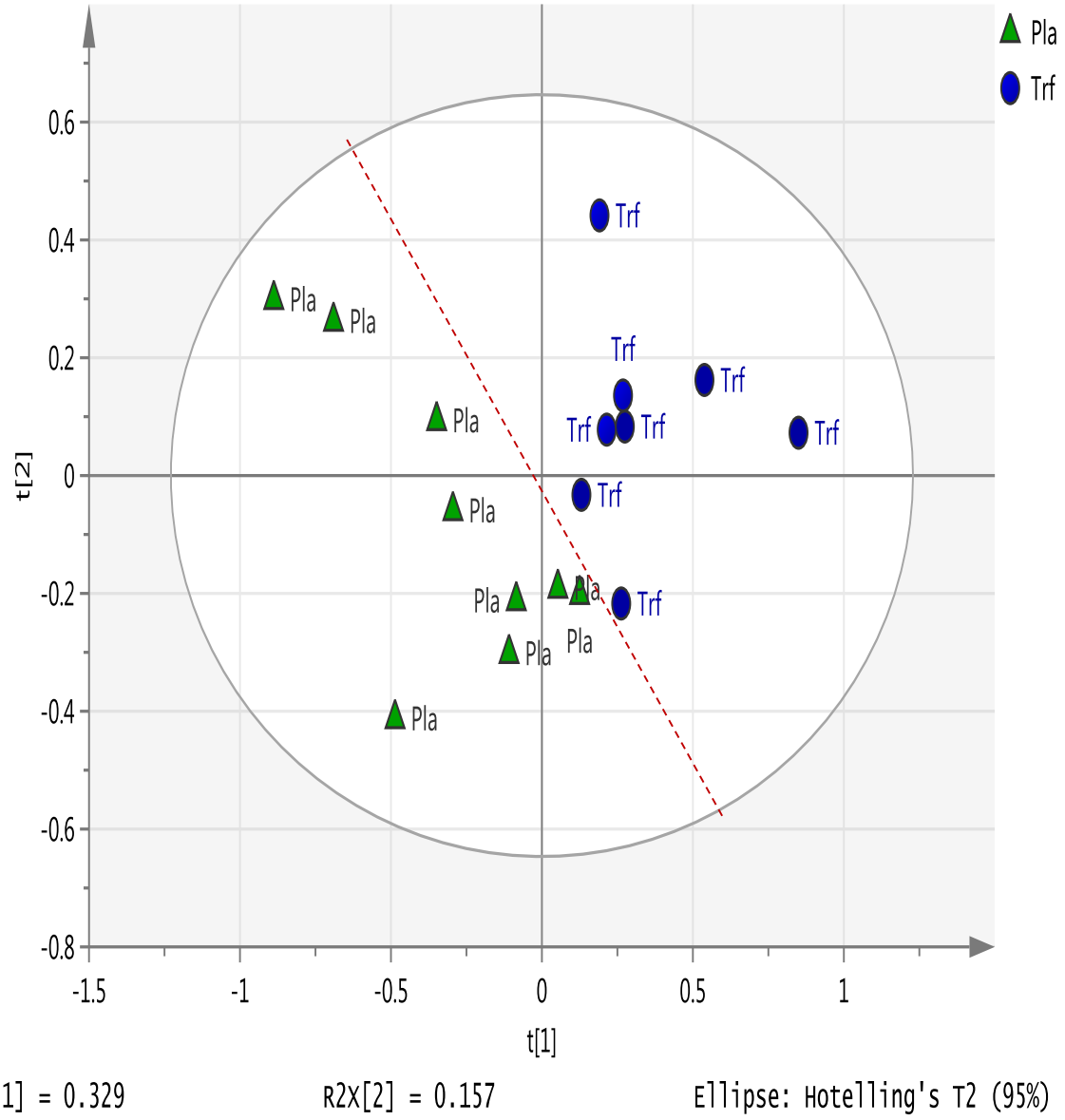
Figure 3-8. PCA score plot from NMR spectra using plasma samples from ESRD patients at Week 12.



PCA- principal component analysis; Pla-placebo; Trf- tocotrienol-rich fraction.

The figure is a depiction of PCA-X score plot at week 12, showing the metabolomic profiles of the same ESRD patients on the placebo and TRF treatment groups which were previously analyzed from baseline. The inside of the eclipse represents 95th percentile of confidence interval and the outside of the eclipse is considered an outlier. The circled in red TRF sample represents an outlier in the PCA.

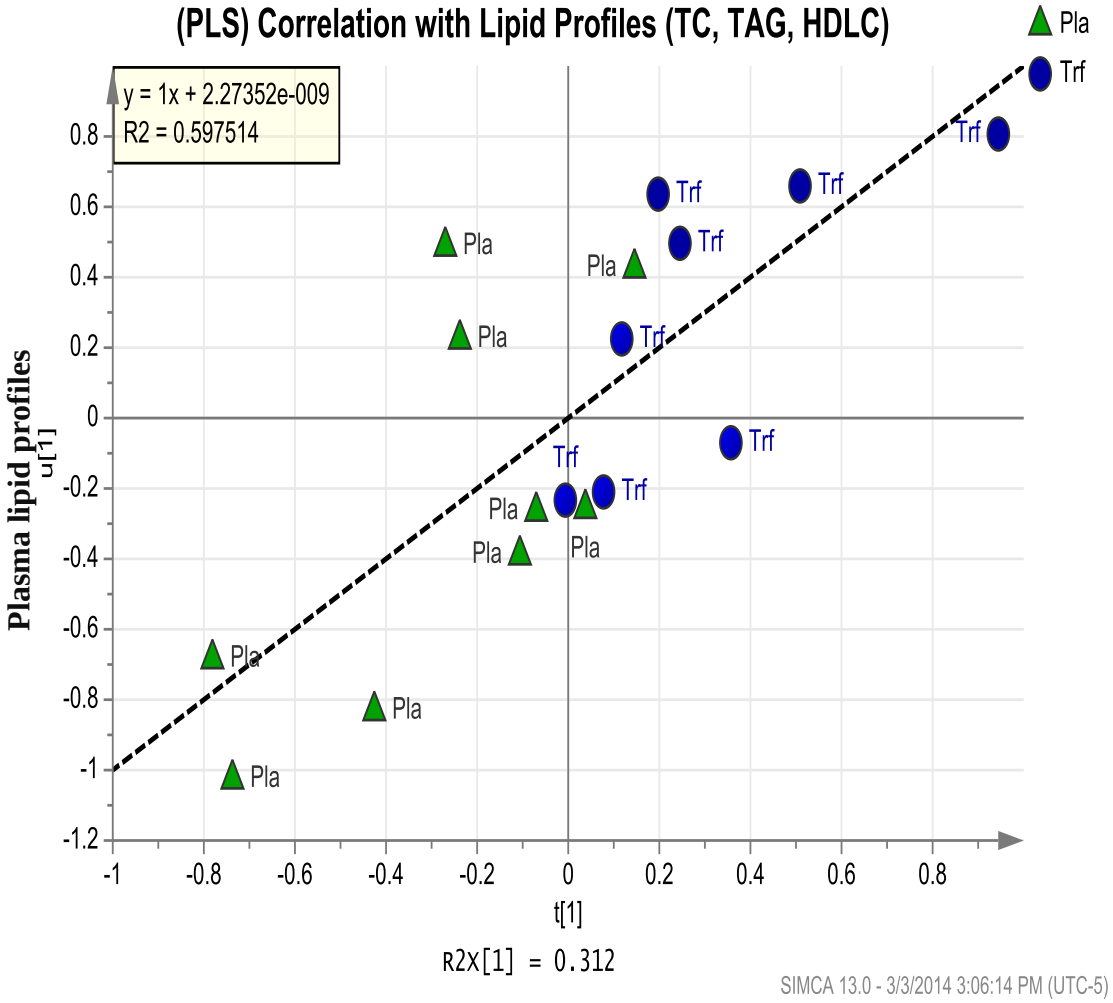
Figure 3-9. PLS-DA score plot from NMR spectra using plasma samples from ESRD patients at Week 12.



PLS-DA partial least square-discriminant analysis; Pla-placebo; Trf- tocotrienol-rich fraction.

The figure is a depiction of PCA-X score plot at week 12, showing the metabolomic profiles of ESRD patients on the placebo and TRF treatment groups. The graph shows a clear separation between the two groups and their metabolites in the plasma.

Figure 3-10. PLS score plot correlating metabolomic profiles and plasma lipids profiles (TC, TAG, and HDL-C) at week 12.

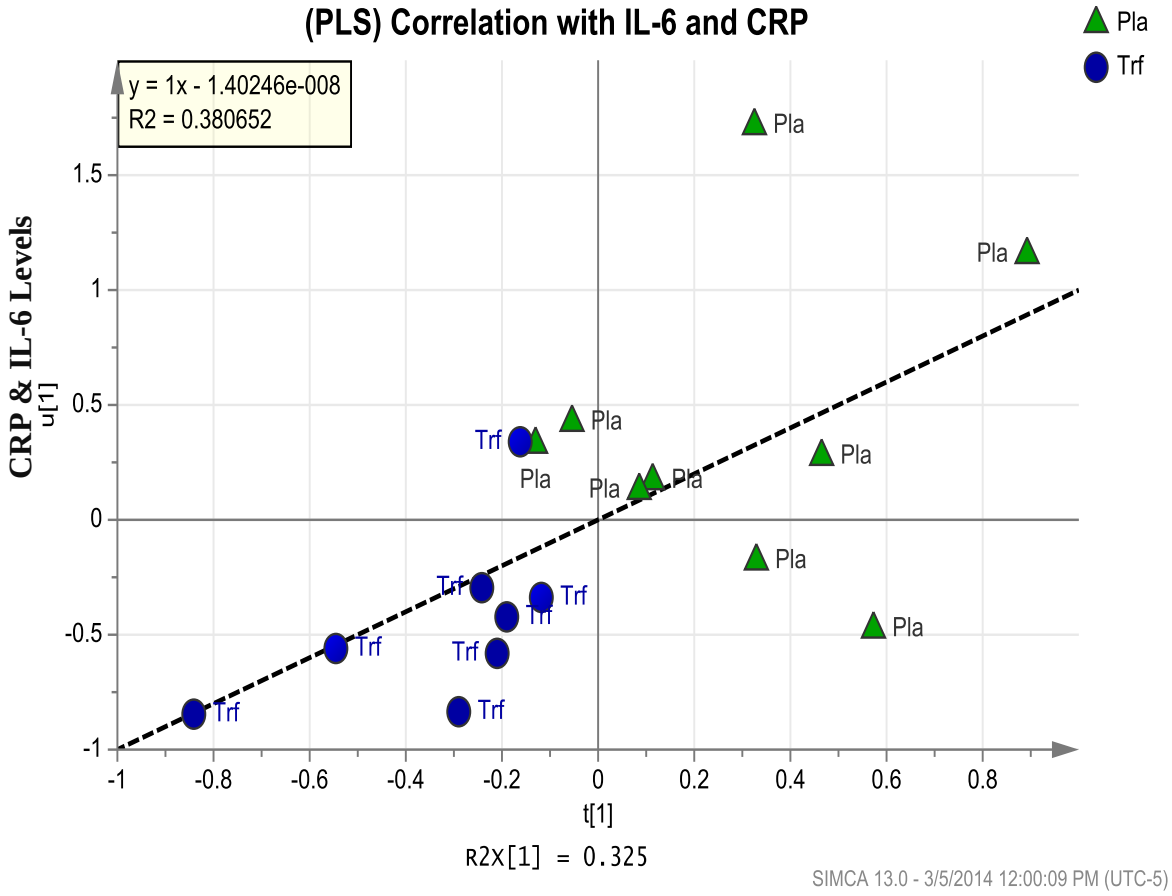


NMR plasma metabolomic profiles

PLS- partial least square; Pla -placebo; TRF- tocotrienol-rich fraction; TC - total cholesterol; TAG -triacylglycerol; HDL-C - high-density lipoprotein cholesterol.

The figure shows PLS score plot, depicting correlation between metabolomic profiles on the (x-axis) and plasma lipid profiles (TC, TAG, and HDL-C) on (y-axis) between the two groups at week 12. The model shows a moderate correlation (R^2 = 0.60) between the metabolomic profiles and the lipid profiles.

Figure 3-11. PLS score plot correlating plasma metabolite profiles to CRP and IL-6 levels.

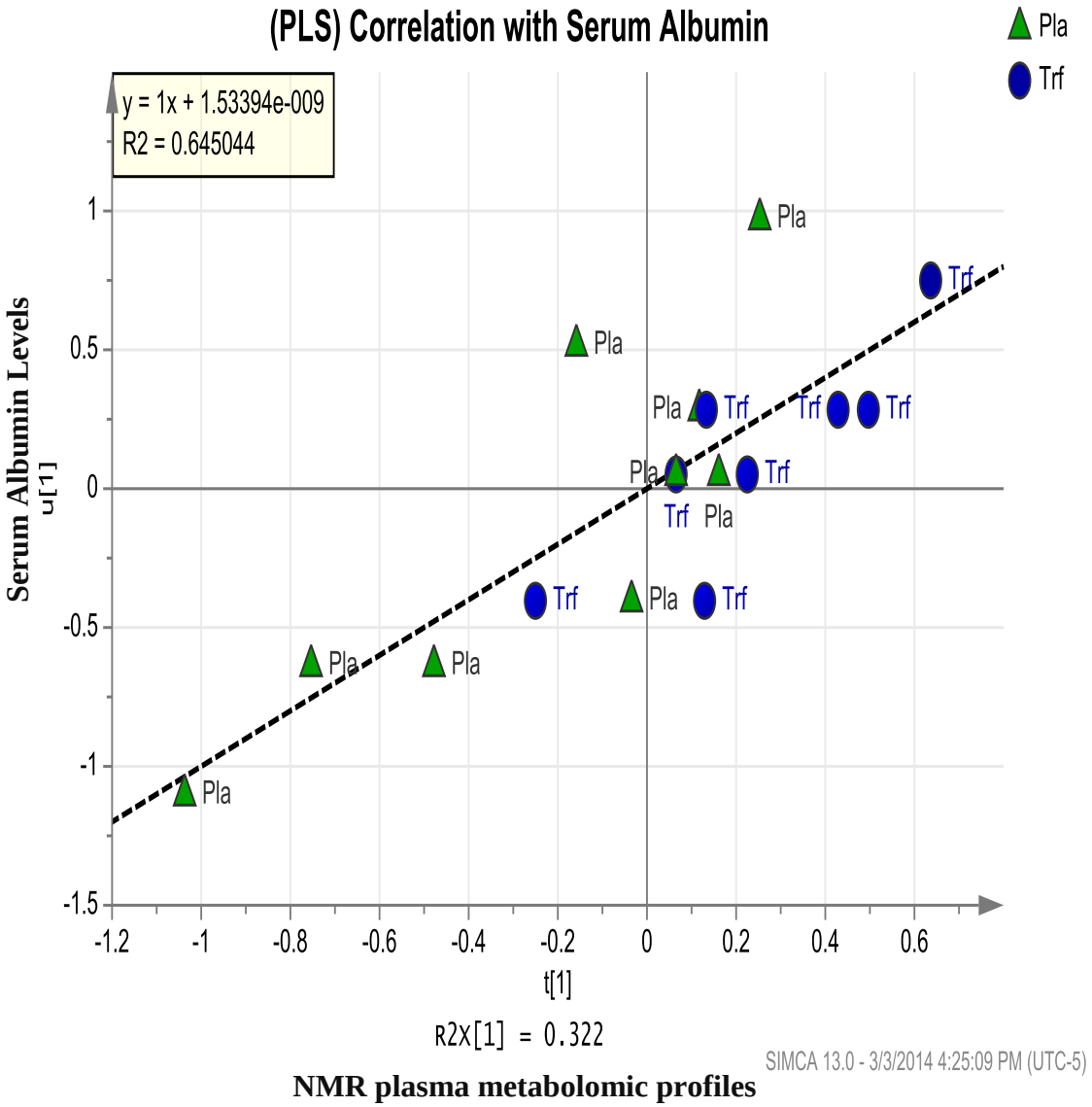


NMR plasma metabolomic profiles

PLS- partial least square; Pla -placebo; TRF- tocotrienol-rich fraction; CRP- C - Reactive Protein; IL-6 – Interleukin 6

The figure shows PLS score plot, depicting correlation between metabolomic profiles on the (x-axis) and CRP/IL-6 on (y-axis) between the two groups at week 12. The model shows a moderate correlation (R^2 = 0.38) between the plasma metabolomic profiles and two the pro-inflammatory markers.

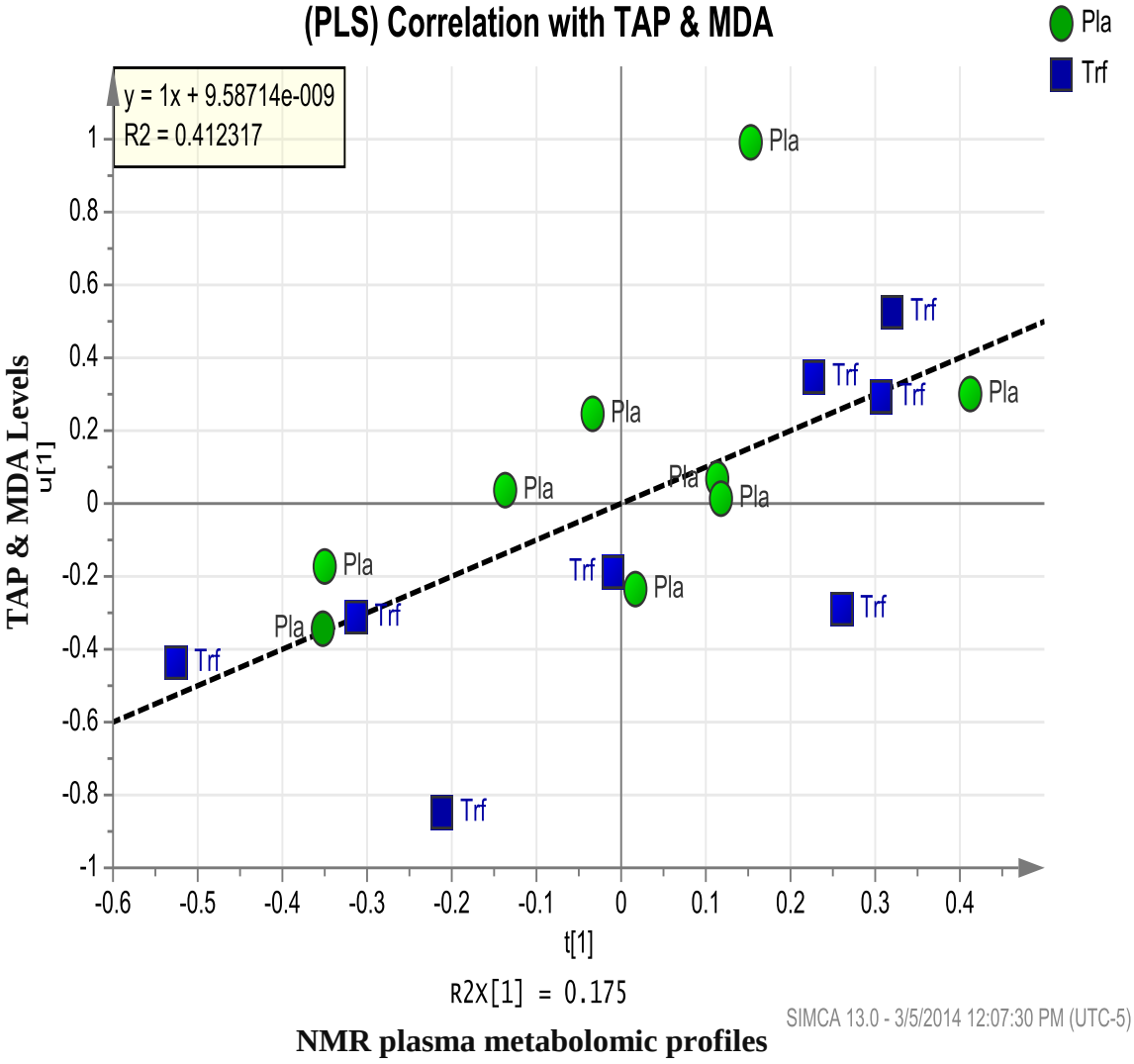
Figure 3-12. PLS score plot, correlation of metabolomic profiles in the plasma and serum albumin at week 12.



PLS- partial least square; Pla -placebo; TRF- tocotrienol-rich fraction

The figure show PLS score plot depicting the correlation between the plasma metabolomic profiles on the (x-axis) and the serum albumin (marker of nutritional status in ESRD patients) (y-axis) at week 12. The results indicate a correlation between the two groups with a (R^2 = 0.65) values.

Figure 3-13. PLS correlation of TAP and MDA compared to metabolomic profiles in the plasma.



NMR plasma metabolomic profiles

PLS- partial least square; Pla -placebo; TRF- tocotrienol-rich fraction;

TAP - Total Antioxidant Power; MDA- Malondialdehyde

The figure show PLS score plot depicting the correlation between the plasma metabolomic profiles on the (x-axis) and the antioxidant (MDA and TAP) activity (y-axis) at week 12. The results indicate a small moderate correlation between the groups with a (R^2 = 0.41) values.

CHAPTER 4

DISCUSSION

CKD patients with ESRD experience an accelerated form of atherosclerosis due to increase in inflammation, increase in oxidative stress and the decrease in cellular antioxidant levels. Therefore by supplementing with 220 mg/day of vitamin E – tocotrienol-rich fraction for 16 weeks, it was hypothesized that this supplement may help reverse and improve oxidative stress, inflammatory markers and increasing the antioxidant activity, in conjunction with improving lipid profiles of HD patients.

The demographic characteristics of the study depict a homogeneous, African American ethnic population in order to reduce variation. Parameters in both groups (placebo and TRF) age, ethnicity, gender, smoking and the clinical parameters distribution were analyzed and demonstrated no significant differences **Table 3-1**, thus any potential comorbidities, and conforming assumption factors influencing the outcome, were minimized. In this study lipid profiles (TAG, TC, LDL-C and HDL-C) were measured for both groups (placebo and TRF) at baseline, week 8, 12 and 16, on which case they were further analyzed for the duration of this intervention for changes in blood plasma. Because ESRD patients are at a high risk for various CVD induce illness, some patients were under the use of statins medications which were approved by their health provider. Additionally the sub-groups of statin users were allowed in this intervention in order to explore any synergistic effects between TRF and statin and whether they affect our measured parameters. Therefore in this study patients were further sub-divided into four additional groups (TRF Non-Statin, TRF Statin, Placebo Non-Statin, and Placebo Statin) **Figure 3-1**, at which point results were analyzed for changes in lipid profiles when groups were

compare against each other **Table 3-2**. The study also analyzed the CETP activity levels in the plasma **Figure 3-2** in order to correlate any changes observed to the lipid profiles. As previously mentioned, this is the first study of its kind, which uses vitamin E-tocotrienols as an intervention supplement on ESRD patients, due to the fact that TRF as a micronutrient contains antioxidant properties that could help fight oxidation, free radicals and obtains lipid lower properties. Also measurements were conducted on NFκB, a protein which is involved in cellular responses, and measurement were taken for CRP as well as IL-6 pro-inflammatory markers. Analysis of lipid profiles was processed using SPSS, and the results were extended to metabolomic analysis, which was conducted using 600 MHz NMR. Furthermore metabolites in the plasma were compared and analyzed using SIMCA-P models, than Chenomx was utilized for quantification/identification of metabolites which changed.

Lipoprotein Profile Effects

One of the most important changes that the study discovered was the decrease in TAG levels from baseline to week 16. An overall gradual decrease in TAG levels was observed over time between TRF Non-Statin (n=23) and TRF Statin (n=17) groups **Table 3-2**. No decreasing trends or patterns were observed over time when looking at the Placebo Non-Statin group, but the Placebo Statin saw sporadic decreases in TAG levels at weeks 8-12 and an increase at week 16. TAG results were also compared in parallel for changes between the sub-groups. It was discovered that during week 8 when comparing TRF Non-Statin to Placebo Non-Statin a significant change in TAG levels was recorded which favored a higher decrease of Placebo Non-Statin. This type of analysis was carried out between the same groups at week 16, and again a significant change was observed but this

time it favored TRF Non-Statin group which recorded a much lower TAG decrease. It is postulated that the reason TAG levels were reduced more on the later weeks during the study might have to do with the fact that TRF supplementation, needs time to build up in order to effect the metabolism pathways of triacylglycerol's. A good visual changes over time in TAG levels between the four sub-groups is observed on **Figure 3-5**. This time course graph show a significant decrease of both TRF Statin and TRF Non-Statin at week 12, at which point this marginal decrease was carried over at week 16. As shown in the graph Placebo Statin observes a clear decreases in TAG levels at weeks 12-16 proving that these medications are also effecting the patients. Therefore is worth postulating that statin drugs are effecting in aiding to change TAG level, as shown by TRF Statin and Placebo Statin results. Statin drugs are known to induce inhibition of HMG-CoA reductase which leads to a decrease of hepatic cholesterol pools and subsequently decreases in production Apo-B contacting VLDL particles and the fact that LDL receptors are up-regulated. VLDL plays a key role in TAG trafficking of lipoprotein in fasting plasma, therefore statins drugs reduce TAG by enhancing clearance of VLDL, and reuptake of LDL occurs via LDL receptors, therefore this would be the path speculated which is imposing a decrease of the TAG and LDL. Another interaction worth speculating as possible affect in the decrease of TAG levels is the synergistic link between statin drugs and TRF supplements which possibly act in inducing inhibition of HMG-CoA reductase **Table 1-3**.

Total cholesterol (TC) levels were also affected, were an overall significant decrease over time is observed in TRF Non-Statin group from start to end, and likewise TRF Statin observed a more gradual decrease until week 12. Additionally Placebo Stain group observed marginal decreases from baseline to week 16, and Placebo Non-Statin

group did not see any significant decreasing patterns **Table 3-2**. Comparison analysis between TRF Non-Statin and TRF Statin (week 16) discovered a significant decrease in TC levels, which favored a bigger decrease in TRF Non-Statin group. This is an important results because it strengthens the notion that TRF supplementation offers lipid altering effects, as recorded in these results, correlating to preview literature with hypocholestrolemic issues were TT exhibits unique lower effect. This could be due to TRF effecting post-transcriptional suppression of HMG-CoA reductase protein, and the enhancement of ubiquitination of HMG-CoA reductase **Table 1-3**.

LDL-C was another altered lipid which observed a decreasing trend, and in this case it was an overall change from baseline to week 16 observed only in TRF Non-Statin group. On the other side TRF Statin group showed a similar gradual decreased in LDL-C level but failed to progress into week 16. Comparison analysis between these two groups showed a significant change in LDL-C favoring a higher decrease in TRF Statin group levels up to week 12, than on week 16 levels increased marginally, whereas Non-Statin TRF showed a significant decrease compared to its counterpart (week 16) **Table 3-2**. Statin Placebo/Non-Statin groups showed small decreases in LDL-C levels which were not significant but both groups like TRF Statin observed in increase at week 16 when comparing levels to week 12.

Last lipid results which the study effected were the HDL-C levels in which case in TRF Non-Statin group observed an increase in levels from baseline to week 16, similar trend was observed in TRF Statin group but only up to week 12. On the other side the Placebo groups whether Statin or Non-Statin did not show any significant increases in HDL-C level, but they were followed by marginal increases throughout the study **Table 3-**

2. A time course comparison between TRF Non-Statin and Placebo Non-Statin showed a significant increase of higher HDL-C levels favoring the TRF group. **Figure 3-6** a change in time of HDL-C levels among the groups depicts the important of supplementing with TRF in ESRD patients who are under Statin medication and or Non-Statin, result show a significant increase ($p<0.05$) in this lipoprotein from baseline to weeks 12 and 16. The increase in HDL-C levels in the plasma, it is postulate could be due to the increased production of HDL particles, the delayed catabolism of HDL particles or the higher cholesterol contents in HDL particles. Another mechanism forcing these results would be the effect of tocotrienols (TT) on the TAG and HDL-C levels at week 12 and 16. Levels for ApoA1 and CETP were also measured in this study although ApoA1 were not discussed in this thesis the levels were significantly higher in the overall TRF groups during week 12 ($p<0.05$), which also coincided with the higher levels of HDLC-C in TRF group's overall [35]. The reason why ApoA1 is important, is because it is a major protein in HDL particle (estimated is 70% of HDL particle), in which aids in clearing fats including cholesterol. Therefore increase in HDL-C levels at week-12 in the TRF groups is explained by the increase in HDL particles which mirrors the increase in ApoA1 at week 12 and this correlation was further solidified by the Pearson's test during weeks 12 and 16 [35]. It is speculate that a link seems to exist in this study were TRF supplementation has significantly affected an increase in HDL-C which was reflected by higher levels of ApoA1 concentration in the plasma. Literature on TT effecting ApoA1 is scarce but a the study by Heng et al [42] does demonstrate the increased expression of ApoA1 precursor when this form of vitamin E (TT) is supplemented.

It worth extrapolating that TRF and TP supplementation, in which case exhibit antioxidant like mechanisms could possibly effect several transcriptional factors such as MAP, PPARα and PPARγ in the up-regulation of proapoA1 [43]. The other marker that was seen to have a lower activity in the overall samples was CETP in TRF group during week 16, in which case it parallels higher HDL-C levels. Significant decreases in CETP levels resulted between Placebo Non-Statin and TRF Non-Statin groups, in which case at week 16 the TRF groups had bigger decreases than its counterpart (placebo) by $P<0.0001$ **Figure 3-2**. A decrease in CETP levels was also seen between Placebo Statin and Placebo Non-Statin comparison, in which case the statin group had a higher decrease than its counterpart even though the sample number are not equivalent in patient number ($p<0.0001$) **Figure 3-2**. CETP plays an important role in lipid metabolism because it mediates the transfer of cholesterol ester from HDL to VLDL remnants in exchange for triacylglycerol's [44]. In this hemodialysis population, patients are experiencing delayed catabolism of TAG rich lipoprotein, so it is speculated that CETP activity may increase in facilitating that TAG-cholesterol transfer between HDL and apoB100 lipoprotein. A recent study made the correlation in HD patients in having higher levels of CETP when compared to control [45] which ultimately is depicted by lower HDL-C levels and higher TAG levels in this population. In our study CETP had significantly decrease and further analysis using Pearson's correlation test between CETP and TAG (a decreased) concentration, at weeks 12 and 16 confirms significant correlation between the two variable. Therefore the second metabolic pathway to consider which improved lipids in decreasing TAG and increasing HDL-C could be CETP mechanisms due to the decrease of its activity levels.

Figure 3-3 explore the changes in the CRP activity between TRF and Placebo groups. It was discovered that CRP levels were significantly lower at baseline compared to weeks 12 and 16. Even though CRP levels increase after baseline it is worth noting that TRF Statin group overall had lower average CRP levels throughout the study. ESRD population has been linked to higher levels of inflammation in the body than normal people, in which case leads to major health issues like CVD's, therefore preferred outcome would be to lower CRP in order to lower inflammation but this study was not able to reduce the CRP levels significantly by supplementing with TRF and or statin use did not affect levels by much. The reason for not achieving this goal could be due to multiple acumen, possible due to higher inter-individual variability in CRP levels, which often is associated with transient inter-current clinical evens and the dynamic response of the human immune system or even the simple fact that CRP fluctuates dramatically upon acute inflammation [46]. In a similar fashion NFκB activity was measured at baseline and week 12. It was observed that the activity of NFκB was significantly lower at baseline when TRF Statin was compared to TRF Non-Statin, but no significances were observed on the other two groups at ether time lines. The same measurement was conducted at week 12 looking for changes between the groups, but no such data was recorded **Figure 3-4**. NFκB which is found in almost all the human cells plays a key role in cellular responses to stimuli of stress, pro-inflammatory agent such as cytokines and CRP. Due to the fact ESRD patients are prone to increased levels of inflammation in their bodies it was postulated that TRF would reverse these effects. The only marginal effect that could be correlated, was the fact that TRF supplementation in conjunction with prescribed Statin drugs seem to marginally help lower NFκB, but only at baseline.

Metabolomic Analysis

Analysis of the plasma metabolomic profiles in HD patients was conducted using SIMCA-P in order to compare and find difference between TRF and placebo group. Unlike previews tests in this part of the study no statin users were included in these analysis. The first analysis conducted was unsupervised PCA-X graph at baseline which found the metabolites being spread throughout the graph which means no correlation between the two groups **Figure 3-7**. Than after PCA was conducted just for week 12 **Figure 3-8** and there was some separation between the two groups unlike in the baseline results. To validate the separation between the two groups PLS-DA model was utilize from the NMR spectra using plasma samples on ESRD patients at Week 12, a discriminate form analysis on which case observe a clear separation between the two groups, which meant a significant difference between TRF and placebo metabolome profile **Figure 3-9**. After establishing a difference between TRF and placebo groups, analysis was conducted using PLS score plot between metabolomic profiles and plasma lipid profiles (TAG, TC, and HDL-C). The results showed a very high correlation as well as separation between the two group exemplified by the significant R^2=0.597 value **Figure 3-10**. These results establish a significant difference in NMR metabolomic profiled of ESRD patients, and their plasma lipid profiles making a fair conclusion that TRF groups have different lipid composition than the placebo groups. The difference in lipid profile in TRF group when compared to placebo is exemplified by the increase of HDL-C, and the decreases observed in TAG and TC levels. We can summarize that there is strong correlation that by supplementing with tocotrienol-rich fractions for 12 weeks the study observed a positive link, in altering the lipid profiles in ESRD population to patients' plasma metabolites.

Additionally PLS plot was also used to analyze the plasma metabolite profiles to CRP and IL-6 level activity in this population. As it was reported previously in the plasma analysis IL-6 and CRP showed no significant changes in effecting any pro-inflammatory markers. In this PLS model we observed moderate difference between the TRF and placebo groups by utilizing two component variable analysis (CRP/IL-6 vs. plasma metabolomic profiles) in which case we observe a small marginal correlation based on R^2=0.38 value. Because of these results on how the TRF and placebo group are observed and clustered **Figure 3-11**, it could be speculated that TRF groups have affected on some degree the inflammatory markers, based on their antioxidant properties and the fact that the TRF group is located at the opposite quadrant on the graph when comparing it to placebo. This maybe none-significant but it would be something worth exploring in future studies with bigger populations as well as statin plus TRF users since this analysis does not factor any statin users. An important analysis by PLS score plot which was discovered, was the correlation of metabolomic profiles in the plasma and serum albumin levels, in which case observed a significant difference and separation between TRF group and Placebo and this is validated by R^2=0.645 **Figure 3-12**. This basically could attribute to TRF protective properties in reducing protein or amino acid degradation, and could be considered a positive link in reduction of inflammation. Last PLS model which was analyzed was the correlation of TAP and MDA in comparison to metabolomic profiles in the plasma **Figure 3-13**. In this case there seems to be a very small correlation non-significant, R^2= 0.41 between two variables and the inner antioxidant molecules which are found in the plasma. Unfortunately there is not a strong link to conclude anything from these finding, this maybe an area for future clinical trial to explored and expand on. Moreover metabolomics profile

analysis in both TRF and placebo does conceives a correlation between inflammatory markers and lipid profiles, suggesting that some plasma metabolites cold predict changes in lipid and inflammatory biomarkers. The comparison and exploration conducted in this thesis was to identify any metabolites of change on ESRD patients using Chenomx (quantification and identification) analysis when comparing TRF and placebo groups NMR spectrums. After our analysis identified roughly 311 metabolites for which patients in both groups TRF and placebo shared. Scrutinizing the data in further detail, when comparing TRF and placebo groups it was discovered that eleven metabolites were categorized to have incurred a significant change in the metabolome profile ($p<0.05$) **Table 3-3**. In this event 8 out 11 metabolites saw a decrease in their expression, and 3 out 11 saw an increase in their expression. Preliminary results of metabolites which changed, after being identified via Chenomx, does reveals that TRF supplementation triggers changes in ESRD, which leading to ether reduction/increase in several biomarkers. The names were identified, as well as the role they play in human metabolism, but currently this data is under further validations before being released to the public.

Conclusion

In the beginning of this study it was hypothesized that by supplementing with tocotrienol rich fraction (TRF) a vitamin E, for 16 weeks in ESRD patients undergoing hemodialysis, it may help to reverse and/or improve; oxidative status, inflammatory markers, increase antioxidants status and improve lipid profiles in this population. Regrettably this postulated hypothesis did not completely come to fluidity. The only portion of the hypothesis which came true was the fact that HD population who have incur ESRD seemed to observe a change and improvement on lipid profiles when using this

micronutrient, as well as when combining it with statin medications. This study also observed a minor link on synergism when combining Statin drugs with TRF, but this remark requires further analysis and validation. Experimentation conducted in regards to reducing oxidative status, inflammatory markers and increase in antioxidant activity, showed the results to be inconclusive, therefore disproving the other portion of this postulated hypothesis.

This clinical intervention using ESRD patients provided conclusive results for future studies in which case, when statin drugs are combined with TRF-vitamin E supplements, and/or TRF Non-Statin alone is given to patients, this seems to improve HD subjects' lipid profiles. A significant decrease of TAG levels was observed as well as an increase in HDL-C was recorded in this clinical study. These results were reflected by the increase in ApoA1 and the decrease in CETP levels Whereas TC and LDL-C observed incremental decreases which need further validation. Using metabolomic analysis further evidence provided that TRF supplementation seemed to have a positive effect in improving and changing lipid profile (TC, TAG, and HDL-C) when compared to placebo. Therefore in this study it was established that TRF supplementation does trigger a change in ESRD metabolome profile.

Even though this study fell short to provide a significant effect from TRF supplementation into improve oxidative, inflammatory and antioxidant status in ESRD patients, various PLS-models using SIMCA-P showed moderate changes and correlations when looking at NMR metabolomic profiles compared to the variables tested (oxidative, inflammatory, antioxidant) in TRF and placebo groups. Correlation between lipid profiles and plasma metabolome shows a significant difference between TRF and placebo groups.

The metabolomic profile analysis in TRF and placebo does display a correlation when looking at inflammatory biomarkers to lipid profiles suggesting that the plasma metabolites could be used to predict changes in biomarkers. Based on the PLS models that we provided, future studies with bigger pool size of subjects maybe need in order to overturn these inconclusive findings.

Future Directions

Future study should focus in validating the eleven metabolites which were found to have significant changes in the TRF metabolome. After identifying them (by name) the focus should turn to the role they play in the human metabolism (metabolic pathways) and how do these metabolites effect ESRD patients in the long run. It is important to see if there are any links between these biomarkers and how they influence inflammation, oxidative status and antioxidant activity levels. Also future studies should definitely focus in establishing a bigger pool size of subjects for these type of clinical interventions, so researchers can reduce errors due to patients' compliance availability, when analyzing the results. Lastly, future studies should focus in establishing whether there is a synergistic link between Statin with TRF supplement when using NMR (metabolomic) as a method of testing.

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ABSTRACT

EFFECTS OF STATIN DRUGS AND TOCOTRIENOL RICH FRACTION SUPPLEMENTATION IN CHRONIC HEMODIALYSIS PATIENTS AND METABOLOMIC PROFILE

by

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Chronic kidney disease (**CKD**) is known as a heterogeneous disorder which currently is on the rise and lately has been classified as a public health issues in the United State and worldwide. CKD is an irreversible and progressive disease which can lead to kidney failure, and this is depicted by the advanced stage of the disorder when it reaches the point, that is classified as end stage of renal disease (**ESRD**) (Stage 5 of CKD) (eGRF <15 mL/min/ 1.73 m^2 working capacity), where both organs are in a total or permanent kidney failure. End-Stage renal disease patients, on hemodialysis have been associated to experience an accelerated form of atherosclerosis, which is induced by inflammation, impairment of antioxidant system and elevated oxidative stress. Since the problem effecting ESRD patients is multifactorial, the objective of this investigation is to explore and look at the effects of supplementing with vitamin E-tocotrienol rich fraction (TRF), a

micronutrient which has anti-inflammatory, antioxidant, and lipid lower capabilities into tackling these comorbid conditions experienced by this population. Therefore the aims of this investigation will be to explore changes in lipid profiles, inflammatory markers, and oxidative status, as well as look at any changes in metabolomic profiles. It was hypothesized that by supplementing with TRF a vitamin E, for 16 weeks in ESRD patients undergoing hemodialysis, it may help reverse and/or improve, oxidative status, inflammatory markers, increase antioxidants status and improve lipid profiles.

The study was double-blinded, randomized, parallel, placebo-controlled design trial, of 81 adult patients undergoing chronic hemodialysis at Great Lake Dialysis Clinic, Detroit MI, where patients routinely received hemodialysis treatments 3 days a week. These HD patients were randomly divided into two different groups before the nutritional intervention began; a placebo group (n=40) and intervention group TRF (n=41). The patients in the TRF group received 2 x 110 mg/day (220 mg/day) of vitamin E tocotrienol (90 mg of TT and 20 mg of TP) whereas the placebo group patients received 2 x 0.68 mg/day of placebo (0.24 mg of TT and 0.44 mg of TP). Blood samples were collected at baseline, week 8, week 12 and 16 in order to perform biochemical analysis of lipid profiles (TC, TAG, LDL-C, and HDL-C), and then after analyze inflammatory markers, measured using enzymatic kits (CRP, NFκB, IL-6). And last part of the study revolved around metabolomics analysis on the plasma samples using (600MHz) NMR technology, than explore changes in metabolomic profile (biomarkers) in ESRD population.

The study recorded changes and improvement on lipid profiles of ESRD patients. A significant decrease of TAG levels was observed as well as an increase in HDL-C was recorded. The change in TAG levels and HDL-C increase, correlated with the decrease in

CETP levels and increase of ApoA1 in the plasma. Other lipid profile like TC and LDL-C observed marginal decreases which were not significant, therefore further detailed analysis is required. In this analysis patients were further divided into four sub-groups to establish links between TRF and statin drugs. It was concluded that based on lipid profiles and metabolomic (NMR) analysis, there seem to be some small correlations of synergism effects when combining Statin drugs with TRF supplements but in order to validate conclusively this minor links, further detailed analysis is required. Study did established that TRF supplementation does trigger a change in ESRD metabolome profile. A correlation between lipid profiles and plasma metabolome shows a significant difference between TRF and placebo groups. Metabolomic profile analysis in TRF and placebo does display a correlation when looking at inflammatory biomarkers to lipid profiles which suggests that plasma metabolites could be used to predict changes in biomarkers. The study did not find any significant changes on inflammatory and oxidative markers. Research into biomarkers of ESRD metabolome when using (Chenomx) did discover and identify eleven biomarkers of importance in human metabolism that observed a significant change due to TRF supplementation when compared to its counterpart placebo group. Therefore in conclusion this investigation has revealed that TRF supplementation does reveal to trigger changes in ESRD metabolome that could be used for disease prediction in HD patients.

AUTOBIOGRAPHICAL STATEMENT

The author Mr. Eno Latifi received his Bachelor and Science Degree from Wayne State University in Detroit, Michigan as part of May 2012 graduating class. While as an undergraduate he volunteer and conducted research for roughly three years in the Nutrition Food and Science department under Dr. Pramod Khosla guidance. Than after upon graduating, to further his studies in the field of nutrition, he returned back in order to work toward and accomplishing in obtaining a Master's and Science Degree from Wayne State University institution.